JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 48

JANUARY 1–~~JULY~~ June 15, 1934

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1934

630.61
N.58j
v.48

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, CHAIRMAN
Chief, Bureau of Chemistry and Soils

F. L. CAMPBELL
Entomologist, Bureau of Entomology

JOHN W. ROBERTS
Senior Pathologist, Bureau of Plant Industry

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
Director of Research, Pennsylvania Agricultural Experiment Station

C. G. WILLIAMS
Director, Ohio Agricultural Experiment Station

L. E. CALL
Director, Kansas Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal: Domestic, $2.25 a year (2 volumes)
Foreign, $3.50 a year (2 volumes)

Single numbers: Domestic, 10 cents
Foreign, 15 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D.C.

361015
S.P

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 48 WASHINGTON, D.C. JANUARY 1–~~JULY~~ JUNE 15, 1934

CONTENTS

	Page
Correlations between Severity of Pruning and Subsequent Growth and Fruit Yield of Apricot Trees. Illus. H. S. REED	1
Correlated Inheritance of Reaction to Diseases and of Certain Botanical Characters in Triangular Wheat Crosses. Illus. ELMER R. AUSEMUS	31
Correlated Inheritance of Reaction to Stem Rust, Leaf Rust, Bunt, and Black Chaff in Spring-Wheat Crosses. H. K. HAYES, E. R. AUSEMUS, E. C. STAKMAN, and R. H. BAMBERG	59
Effect of Washing on the Keeping Quality of Hens' Eggs. Illus. REECE L. BRYANT and PAUL FRANCIS SHARP	67
A Morphological Study of Blind and Flowering Rose Shoots, with Special Reference to Flower-Bud Differentiation. Illus. D. S. HUBBELL	91
Zenillia libatrix Panzer, a Tachinid Parasite of the Gypsy Moth and the Brown-Tail Moth. Illus. PHILIP B. DOWDEN	97
Development of a Standard Cage Method for Testing the Effectiveness of Stomach-Poison Insecticides on the Japanese Beetle. Illus. WALTER E. FLEMING	115
Frog-Eye (*Cercospora diazu* Miura) on Stems, Pods, and Seeds of Soybean, and the Relation of these Infections to Recurrence of the Disease. Illus. SAMUEL G. LEHMAN	131
Varietal Differences in Cotton Boll Shedding as Correlated with Osmotic Pressure of Expressed Tissue Fluids. Illus. R. S. HAWKINS, S. P. CLARK, GEO. H. SERVISS, and CHAS. A. HOBART	149
Border Effect in Irrigated Plots of Marquis Wheat Receiving Water at Different Times. Illus. D. W. ROBERTSON and DWIGHT KOONCE	157
The Influence of Phosphorus Deficiency in Dairy Cows on the Coefficient of Digestibility and the Balance of Calcium and Phosphorus. W. H. RIDDELL, J. S. HUGHES, and J. B. FITCH	167
A Study of the Iodine Content of Pennsylvania Potatoes. Illus. DONALD E. H. FREAR	171
Comparative Value of Some Commercial Protein Supplements in the Rations of Growing Chicks. Illus. O. JOHNSON and D. BRAZIE	183
Studies on *Armillaria mellea* (Vahl) Quel., Infection, Parasitism, and Host Resistance. Illus. HAROLD E. THOMAS	187
Determination of Hardiness in Alfalfa Varieties by Their Enzymatic Responses. Illus. H. M. TYSDAL	219
Cutting Yields of Hogs an Index of Fatness. Illus. K. F. WARNER, N. R. ELLIS, and PAUL E. HOWE	241
Physical Characteristics of Hog Carcasses as Measures of Fatness. Illus. O. G. HANKINS and N. R. ELLIS	257
Factors Affecting *Gladiolus* in Storage. Illus. J. I. LAURITZEN and R. C. WRIGHT	265
Developmental Anatomy and Homologies in Wheat. Illus. M. A. MCCALL	283
Self-Fertilization in Sugar Beets as Influenced by Type of Isolator and Other Factors. Illus. H. E. BREWBAKER	323
Maize Crossing Values in Second-Generation Lines. Illus. ROBERT L. DAVIS	339
Hyperparasitism in the Case of Some Introduced Lepidopterous Tree Defoliators. A. B. PROPER	359
The Relation of "Dark Center" to the Composition of Rutabagas. E. B. HOLLAND and C. P. JONES	377
Size, Shape, and Orientation of Plots and Number of Replications Required in Sweetpotato Field-Plot Experiments. Illus. ROSS C. THOMPSON	379
Uniformity in Pathogenicity and Cultural Behavior among Strains of the Cabbage-Yellows Organism. L. M. BLANK	401

Page

Toxin Produced by *Bacterium tabacum* and Its Relation to Host Range. Illus. E. E. Clayton ... 411

Characteristics of the Piroplasms *Babesia argentina* and *B. bigemina* in the United States. Illus. Charles W. Rees ... 427

The Calcifying Properties of Green, Artificially Dried, and Sun-Cured Pasture Herbage. R. E. Hodgson and J. C. Knott ... 439

Injury from Calcium Arsenate-Hydrated Lime Spray on Snap Beans Retarded in Growth by Unfavorable Soil Conditions. Illus. Loyd W. Brannon ... 447

The Effect of Hypertonic Sugar Solutions on the Thermal Resistance of Bacteria. Illus. A. C. Fay ... 453

The Influence of Lime on the Reaction of Subsoils. A. W. Blair and A. L. Prince ... 469

Growth and Injurious Effects of *Cronartium ribicola* Cankers on *Pinus monticola*. Illus. H. G. Lachmund ... 475

Run-Off and Erosion from Plots of Different Lengths. Illus. F. L. Duley and F. G. Ackerman ... 505

The Absorption and Evaporation of Moisture from Plant Containers. Illus. Linus H. Jones ... 511

The Relation between Abnormal Orientation of the 4-Day Embryo and Position of the Chick at Hatching. Illus. J. R. Cavers and F. B. Hutt ... 517

Colorado and Virginia Strains of Codling Moth in Relation to Their Ability to Enter Sprayed and Unsprayed Apples. Illus. Walter S. Hough ... 533

The Nutritive Value of the Proteins of Alfalfa Hay and Clover Hay When Fed Alone and in Combination with the Proteins of Corn. Kenneth L. Turk, F. B. Morrison, and L. A. Maynard ... 555

Relation of Length of Day to Growth of Timothy. Illus. Morgan W. Evans and H. A. Allard ... 571

Distribution of Oxygen and Carbon Dioxide in Mushroom Compost Heaps as Affecting Microbial Thermogenesis, Acidity, and Moisture Therein. Illus. Edmund B. Lambert and A. C. Davis ... 587

Irregularities in the Inheritance of the Hairy-Neck Character Transposed from *Secale* to *Triticum*. Illus. J. W. Taylor ... 603

The Comparative Effectiveness, in the Dairy Ration, of Supplements of Phosphorus in the Form of Orthophosphoric Acid, Monosodium, Disodium, Trisodium Phosphates, and Bone Meal. Illus. William A. Turner, Edward B. Meigs, Edward A. Kane, Leo A. Shinn, and Walter S. Hale ... 619

Inheritance of Resistance to Loose Smut in Certain Wheat Crosses. Illus. D. C. Tingey and Bion Tolman ... 631

The Manganese Content of Grasses and Alfalfa from Grazed Plots. Donald W. Bolin ... 657

Plant-Tissue Relations of the Sugar-Beet Curly-Top Virus. Illus. C. W. Bennett ... 665

Chemical Composition and Yield of the Alaska Pea as Influenced by Certain Fertilizers and by the Stage of Development. Samuel L. Jodidi and Victor R. Boswell ... 703

Abnormalities in the Flower and Fruit of *Capsicum frutescens*. Illus. H. L. Cochran ... 737

Soil Treatment in Relation to Clubroot of Cabbage. R. H. Larson and J. C. Walker ... 749

Life History of the Crown-Gall Organism in Relation to Its Pathogenesis on the Red Raspberry. Illus. W. M. Banfield ... 761

Chromosomes in Hybrids between *Euchlaena perennis* and *Zea mays*. Illus. A. E. Longley ... 789

A Bacterial Disease of *Hedera helix*. Illus. Richard P. White and Lucia McCulloch ... 807

Growth of Chickens as a Function of Feed Consumption. Illus. Harry W. Titus, Morley A. Jull, and Walter A. Hendricks ... 817

Comparison of the Pullorin and the Rapid Whole-Blood Agglutination Tests for Pullorum Disease. Hubert Bunyea ... 837

The Distribution and Condition of Nitrogen in Three Horizons of a Differentially Fertilized Hagerstown Clay Loam Soil Planted to Apple Trees in Metal Cylinders. Walter Thomas ... 845

Page

Life History of the Hairy-Root Organism in Relation to Its Pathogenesis on Nursery Apple Trees. Illus. E. M. HILDEBRAND 857

Seasonal Development of Hairy Root, Crown Gall, and Wound Overgrowth on Apple Trees in the Nursery. Illus. A. J. RIKER and E. M. HILDEBRAND 887

Hairy Root, Crown Gall, and other Malformations at the Unions of Piece-Root-Grafted Apple Trees and Their Control. Illus. A. J. RIKER, G. W. KEITT, E. M. HILDEBRAND, and W. M. BANFIELD 913

Technic for Obtaining Spermatozoa for Physiological Dairy Studies and Artificial Insemination. Illus. FRED W. MILLER and EVERETTE I. EVANS 941

Aphelenchoides xylophilus, N. Sp., a Nematode Associated with Blue-Stain and Other Fungi in Timber. Illus. G. STEINER and EDNA M. BUHRER 949

Relation of Barberry to the Origin and Persistence of Physiologic Forms of *Puccinia graminis*. Illus. E. C. STAKMAN, M. N. LEVINE, RALPH U. COTTER, and LEE HINES 953

Size and Arrangement of Plots for Yield Tests with Cultivated Mushrooms. Illus. EDMUND B. LAMBERT 971

Magnesium, Calcium, and Iron Requirements for Growth of *Azotobacter* in Free and Fixed Nitrogen. Illus. C. KENNETH HORNER and DEAN BURK 981

The Normal Development of the Leg Bones of Chickens with Respect to Their Ash Content. Illus. H. M. HARSHAW, J. C. FRITZ, and HARRY W. TITUS 997

The Lethal Effect of Low Temperatures on the Various Stages of the Confused Flour Beetle. Illus. ROY H. NAGEL and HAROLD H. SHEPARD 1009

Increase of Kernel Weight in Common Wheat Due to Black-Point Disease. L. R. WALDRON 1017

A Statistical Study of the Relationships between the Constituents of Milk. Illus. ALEX BLACK and LEROY VORIS 1025

Influence of Calcium Phosphorus Intake on Bovine Blood. J. E. GREAVES, E. J. MAYNARD, and WENDELL REEDER 1033

Survival of Blister-Rust Mycelium in Western White Pine. H. G. LACHMUND and J. R. HANSBROUGH 1043

Effect of Leaf Rust (*Puccinia triticina*) on Yield, Physical Characters, and Composition of Winter Wheats. Illus. RALPH M. CALDWELL, H. R. KRAYBILL, J. T. SULLIVAN, and LEROY E. COMPTON 1049

Inheritance of Resistance to Loose Smut and Covered Smut in Some Oat Hybrids. Illus. T. R. STANTON, GEORGE M. REED, and F. A. COFFMAN 1073

Testing Alfalfa for Resistance to Bacterial Wilt. FRED REUEL JONES 1085

A Gall Similar to Crown Gall, Produced on *Gypsophila* by a New *Bacterium*. Illus. NELLIE A. BROWN 1099

Experiments on Iarovizing Corn. Illus. GEORGE F. SPRAGUE 1113

The Microbial Decomposition of Successive Cuttings of Alfalfa Hay under Aerobic Conditions. Illus. E. A. BEAVENS and L. H. JAMES 1121

Press Fluid from Heated Beef Muscle. Illus. ALICE M. CHILD and MARY BALDELLI 1127

Carbon Dioxide Formation by Clean and Scabby Potatoes. Illus. B. F. LUTMAN 1135

ERRATA AND AUTHORS' EMENDATIONS

Page 178, line 6, "are presented" should be "are not presented."

Page 236, the curve in figure 3 should contain only five points, as indicated in text.

Page 274, line 2, before "Lucette" insert "Alice Tiplady,".

Page 304, figure 11, omit black dot at right in *A* and *G*.

Page 309, figure 13, new drawing furnished to correct errors in *A* and *C*.

Page 336, last line under "Discussion", insert "no" before "appreciable."

Page 429, sixth line from bottom, "2.26±0.04" should be "5.00±0.05" and "5.00±0.05" should be "2.26±0.04."

Page 513, table 3, item 7, "(413.–33390.75)" should be "(413.33–390.75)."

Page 712, table 4, last column, sample 17, "8.82" should be "3.82."

Page 719, table 6, last column, average of samples 13 and 14 (fresh weight), ".073" should be ".079."

Page 732, table 11, next to last column, average of samples 7 and 8, "3.33" should be "2.33."

Page 737, seventh line from bottom, "vasal" should be "basal."

Page 794, eighth line from bottom, "no. 5" should be "no. 6."

Page 799, table 4, column 1, third line from bottom, and column 3, bottom line, "*Z. mays* (F_2)" should be "*Z. mays*×(F_2)."

Page 812, sixteenth line from bottom, "nor in agar tubes" should be "and no growth occurred in shake agar tubes."

Page 830, footnote 4, after last equality sign, "$_0.9692$" should be "0.9692."

Page 840, table 2, column 4, reaction for fowls 74, 10, and 90 should be shown as positive.

Page 918, legend for figure 1, asterisk (*) should be "°".

Page 1011, first line, omit sentence beginning "Although a few points."

Page 1105, under "Beef-infusion agar slants", second and third sentences should be combined to read, "Under the hand lens it has a metallic luster on beef agar, pH 6.8, at temperatures of 25° to 30° C."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 48 WASHINGTON, D. C., JANUARY 1, 1934 NO. 1

CORRELATIONS BETWEEN SEVERITY OF PRUNING AND SUBSEQUENT GROWTH AND FRUIT YIELD OF APRICOT TREES [1]

By H. S. REED [2]

Plant physiologist, California Agricultural Experiment Station

INTRODUCTION

The question often asked, "Does severe pruning really benefit the fruit tree?" has seldom been answered definitely, although scores of experiments have been undertaken with the expectation of getting information on this point. Many of these experiments were begun with young trees and were terminated before enough time had elapsed to make the tests really valid. Young trees, free from serious competition in the root zone, usually grow and produce good fruit without much reference to the system of pruning employed. The important question is the effect of pruning when in later years competition becomes severe between the trees both above and below the surface of the soil.

Another class of experiments has been seriously handicapped by the conditions resulting from a period of rather free growth due to a lack of pruning before the experiment was initiated. The profound disturbance of the physiological balance of trees that were heavily pruned after having made unrestricted growth introduced a large, but indeterminate, variable into the problem.

This paper contains an analysis of the pruning results based largely upon the coefficients of gross and partial correlation between the amount of wood removed, the growth of the tree, the amount of fruit produced, and the average number of fruits per pound. The records were obtained from an orchard of 280 Royal apricot trees on which the writer conducted experiments in pruning for 16 years.

PLAN OF THE EXPERIMENTS

The orchard consisted of 56 plots in 4 replications of 14 plots each, planted for the pruning experiment. Each replicated plot contained 5 trees. The arrangement of these plots, to which 10 systems of pruning were applied, is shown in figure 1. Plots 1 to 4 were pruned both in the summer and in the winter; plots 5 to 10 were pruned in

[1] Received for publication June 10, 1933; issued February 1934. Paper no. 284, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station.

[2] Grateful mention is made of the assistance of J. Enderud in conducting the experiments and preparing the illustrations for this paper, and also of that of Hannah D. Reed in computing most of the coefficients of correlation and in compiling data.

the winter only. The types of pruning and the plots on which they were applied were as follows:

Plots pruned summer and winter: 1, central-shaft type, severely pruned; 2, central-shaft type, moderately pruned; 3, vase type, severely pruned; 4, vase type, moderately pruned.

Plots pruned in winter only: 5, central-shaft type, severely pruned; 6, central-shaft type, moderately pruned; 7, vase type, severely pruned; 8, vase type, moderately pruned; 9, vase type, ringed; 10, lightly pruned type, moderately pruned.

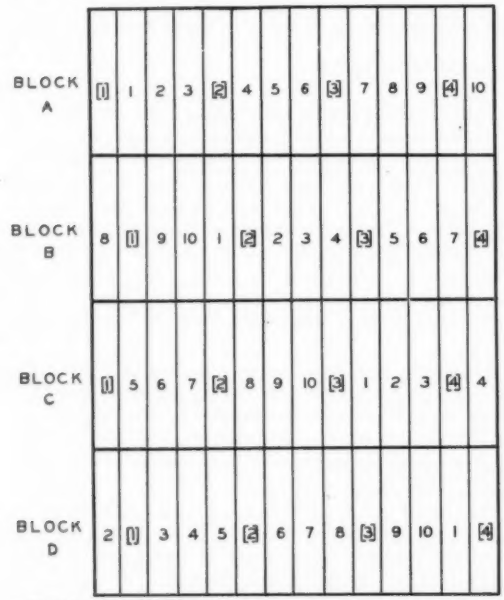


FIGURE 1.—Plan of orchard used for pruning experiments. Numbers in brackets indicate control plots, in which trees were unpruned so far as possible. Numbers without brackets indicate plots on which different types of pruning were employed.

On the four control plots in each block the trees were pruned only enough to allow the passage of implements of cultivation, or to remove broken branches.

There were no trees adjacent to the ends of the plots in blocks A and D (fig. 1). Nevertheless the freedom from competition affected each of the 14 plots similarly.

There were trees adjacent to the other two sides, viz, pear trees adjoined plots A10, B[4], C4, and D[4] until February 1924; subsequently orange trees were planted. Adjacent to plots A[1] and B8 there was a row of apple trees, later replaced by citron trees. Adjacent to C[1] and D2 there were apricot trees of the same age and variety as those under experiment.

Field experiments with horticultural crops are admittedly difficult to interpret because there are usually so many unmeasured factors in the equation. After the experimenter has been careful to measure, weigh, analyze, and correlate his data, there are still other, less ponderable, factors which elude his comprehension. The experimenter is fortunate if these more elusive factors affect all his plots to a somewhat similar extent. Then by the laws of chance there will be approximately the same number of plus as minus errors, and they will tend to offset each other, provided a sufficiently large population of trees has been employed in the experiment. In any case the statistical reliability of the data should be tested by means of the probable error of whatever measurements the experimenter used in judging results.

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND INCREMENT IN AREA OF THE CROSS SECTION OF THE TRUNK

VARIABILITY IN WEIGHTS OF WOOD REMOVED

The weight of wood removed will be regarded as the independent variable because it is the factor experimentally varied. The correlations presented in the following pages show the amount of relationship between the above-mentioned variable and others which may be considered as dependent variables.

The weight of wood removed was recorded for all trees immediately after the annual pruning during the dormant season. The weight of the succulent shoots removed in summer from plots 1, 2, 3, and 4 was not recorded because it was not comparable to the weight of the woody shoots removed in winter. For this reason, separate computations were generally made on the four plots which received summer and winter pruning. The records of the size of trees in 1927 were unfortunately lost soon after they were taken.

The amount of wood removed at the winter pruning varied greatly because certain plots were intentionally severely pruned, as stated above. A frequency polygon would show an asymmetrical distribution of the population if classified according to severity of pruning. For example, the average weight of prunings per tree in 1930 was 27.66 pounds; the standard deviation was 25.94 pounds. This great variability is due to the wide range in the individual weights of the prunings, namely, 7 to 157 pounds. It may be considered that the amount of wood removed depended upon the type of pruning employed, the judgment of the man who pruned the trees, the size of the tree, the amount of growth made in the preceding year, and the necessity of forming a framework which could support the weight of new vegetation and fruit. The weight of wood removed is given in table 1.

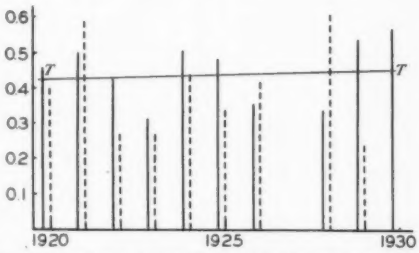


FIGURE 2.—Correlations between weight of wood removed by pruning (w) and increase in area of cross section of trunk (i) in the succeeding season. Continuous lines represent coefficients of plots 1 to 10; broken lines those of plots 1 to 4; T T, line of trend of coefficients of plots 1 to 10.

The growth of the trees was computed from measurements of the circumference of the trunks taken annually. The area of the cross section of the trunk has been found to be a satisfactory index of the size of the tree.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

The coefficients of correlation for weight of prunings (w) and increment in growth of the tree trunks (i) in the subsequent growing season for individual trees in all 10 plots which were pruned, are shown in figure 2. The broken lines represent the same relationship for individual trees in the four plots which were pruned in the summer as well as in the winter. The coefficients representing the gross correlations for all plots are positive and statistically significant, since the probable errors ranged from ± 0.036 to ± 0.045.

TABLE 1.—*Average weight of wood removed by the winter pruning (pounds per tree),[a] 1922 to 1931*

Plot no.	1922	1923	1924	1925	1926	1928	1929	1930	1931
TREES PRUNED SUMMER AND WINTER									
1	59.5±3.64	39.1±2.23	50.4±3.20	67.7±3.67	43.6±3.92	39.0±3.28	45.0±4.78	11.1±0.87	20.4±2.04
2	62.2±2.56	35.6±1.55	52.1±1.67	79.2±2.68	57.6±2.13	42.0±2.57	39.6±2.40	13.1±1.04	19.5±1.50
3	62.9±1.72	44.1±2.18	49.0±1.46	80.5±2.23	56.6±2.25	36.3±2.27	43.8±2.36	10.8±0.88	20.1±1.12
4	65.5±2.15	39.1±1.63	45.8±1.16	74.2+2.11	51.3±1.42	37.6±2.12	48.8±2.72	11.9±0.67	19.9±1.31
TREES PRUNED IN WINTER									
5	71.6±2.76	49.9±2.32	66.0±2.36	80.5±2.41	80.2±2.74	60.6±3.65	84.4±4.48	37.8±2.50	59.8±4.67
6	72.7±2.31	45.1±1.85	68.6±2.49	83.5±2.61	76.0±2.45	65.0±4.02	87.0±4.49	38.8±3.92	53.5±2.67
7	78.3±2.35	62.6±3.46	79.3±2.88	84.1±3.17	83.2±2.55	68.7±3.57	89.6±5.04	42.2±3.92	65.7±4.09
8	80.4±3.00	54.2±3.51	86.9±4.15	110.2±4.68	91.1±3.18	83.8±6.13	123.7±9.02	52.2±5.27	78.0±5.41
TREES GIRDLED									
9	70.6±3.56	40.7±2.72	71.7±3.45	84.4±3.03	78.8±3.22	54.0±3.03	75.5±5.74	29.3±1.91	54.8±3.64
TREES NOT HEADED BACK, MODERATELY PRUNED									
10	41.9±2.52	53.5±4.28	91.4±3.74	64.5±2.02	36.1±2.06	20.9±1.73	35.1±3.04	18.5±1.45	32.1±3.00
CONTROL TREES									
A[1], B[2], C[3], D[4]	57.9±2.98	9.5±1.60	24.2±2.19	37.5±2.98	15.9±2.13	52.6±3.18	10.3±0.87	4.9±0.66	20.8±1.83

[a] The records for 1927 were lost soon after they were taken.

The trend of the coefficients of correlation is shown in figure 2 by the line $T\ T$. Its equation, determined by the method of least squares, is

$$y = 0.421 + 0.003x$$

The root-mean-square deviation of observed from calculated values is 18 percent of the mean and may be considered as satisfactory, considering the number of observations available. The trend indicates that there would be no material change in the coefficients of correlation, at least for some time.

The nature of the correlation may be illustrated by the results for 1925. Figure 3 shows the regression line of growth of tree trunks on weight of wood removed for 1925. The distribution of the means of y for each class of x is essentially linear and passes through the point P which is at the intersection of the lines representing the means of X and Y. The equation to the line as drawn is

$$y = 11.87 + 0.237x$$

This speaks strongly for the linearity of the regression and the validity of using r, the coefficient of gross correlation, as a measure of the association of the two variables.

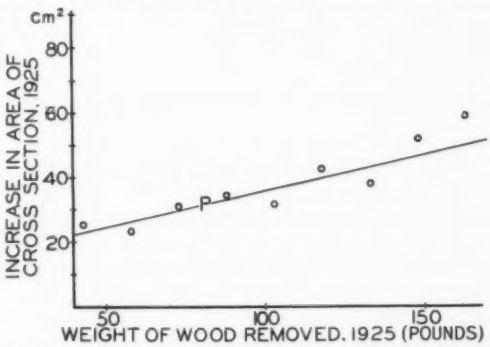


FIGURE 3.—Regression line for means of i for each class of w for 1925, calculated from the equation $y = 11.87 + 0.237x$. Circles represent observed values of i.

So far as these computations go they indicate that the pruning of these trees was favorable to their growth and that the greater growth followed the more severe pruning.

The broken lines in figure 2, which represent the coefficients of correlation for plots 1 to 4, which were pruned in the summer as well as in the winter, agree fairly well with the lines for the other set of plots except for the last 3 years. The coefficients of gross correlation show that there was a marked tendency to greater trunk growth on those trees from which more wood was removed. The natural inference that growth tended to increase with severity of pruning is not established, however, until the variation in tree size is taken into account, because the amount of wood removed depends on both severity of pruning and size of tree. There might be a high correlation between wood removed and following growth where only one uniform type of pruning was employed, provided there were great variability in size of tree and in rate of growth with uniform pruning, because of accidental factors such as soil variability. The weight of wood removed from a large tree was generally greater than the weight of wood removed from a smaller tree, even when both were pruned with the same relative severity.

The coefficients of correlation should therefore take into account the size of the trees at the time they were pruned. If we obtain the coefficients of partial correlation between weight of prunings, increment in the area of the cross section of the trunk and that actual area of the cross section at the time of pruning the tree, we may obtain the values of $r_{wi \cdot a}$, the coefficients of correlation between weight of wood removed and increase in the area of the cross section of the trunk; assuming that the trees when pruned all had the same area of cross section (table 2). The coefficients of partial correlation are small in comparison with their probable errors and are therefore without statistical significance. We cannot conclude from them that the amount of wood removed had an important influence upon the growth of the trunks.

TABLE 2.—*Partial correlation coefficients, $r_{wi \cdot a}$, for the correlations between weight of wood removed by pruning and increments in area of cross section, assuming that all trees were equal in size*

Year	$r_{wi \cdot a}$	Year	$r_{wi \cdot a}$	Year	$r_{wi \cdot a}$
1920	0.031±0.048	1924	0.017±0.048	1928	0.018±0.048
1921	.013± .048	1925	.021± .048	1929	.032± .048
1922	.030± .048	1926	.009± .048	1930	.035± .048
1923	−.004± .048				

AVERAGE SIZE OF TREES EXPRESSED AS A PERCENTAGE OF THE ADJACENT CONTROL PLOTS

We may proceed to investigate another aspect of the problem, namely, "How do the trees in these plots compare in size with the nearest unpruned controls?" This is not a difficult comparison to make because every fourth plot was a control.

If C_1 and C_2 were the average sizes of the trees in the two control plots, then the theoretical sizes for the intervening plots are—

$$p_1 = \frac{3C_1 + C_2}{4}$$

$$p_2 = \frac{2C_1 + 2C_2}{4}$$

$$p_3 = \frac{C_1 + 3C_2}{4}$$

The size of the trees in the 10 plots was computed as a percentage of the adjacent controls by these formulas for 1924 and for 1931 (table 3). The former followed a small crop in which the trunk growth was relatively large; the latter followed several large crops of fruit during which time the trees made less growth than formerly.

One can see that the average area of cross section of these trees became more uniform as the trees grew older. While there were 4 plots in 1924 in which the sizes were distinctly smaller than in the adjacent control plots in 1931, 3 of these 4 plots contained trees whose deviations were ±5 percent or less, leaving only 1 plot with a deviation greater than 10 percent from the computed size.

TABLE 3.—*Area of cross section of trees in various plots expressed as a percentage of the nearest unpruned control plots*

Time of pruning and plot no.	Year	
	1924	1931
Pruned summer and winter:		
1	85	85
2	95	90
3	92	96
4	100	98
Pruned only in winter:		
5	87	95
6	86	96
7	87	98
8	104	108
9	93	98
10	98	107

The reader will likely be impressed (1) by the fact that the average sizes in 8 of the 10 plots, in each year for which data are given, are less than 100 and (2) that most of the deviations are not very great, especially in 1931. The direction of the deviations is, therefore, toward the minus rather than toward the plus side. Have these deviations any significance? If we assume that in an unlimited population the deviations would be zero, then the chance of a deviation of as great as 8:2, or greater, is only 1:17+ (that is, if pruning is really uncorrelated with growth, 8 or more minus differences are expected only about once in 17 times).

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND YIELD OF FRUIT

The biologist with his interest in the functions of living things, as well as the horticulturist with his interest in the production of orchard fruits, is deeply concerned with this problem. Biologists have investigated the relations of vegetative and reproductive activities of many organisms, the effect of checking one of these activities, and the question of an equilibrium between them. Horticulturists have investigated the question of pruning in relation to the quality of fruit, periodic bearing, the effect of pruning at certain stages of growth, the effect of reducing the number of growing points, and other factors. Few of these problems have been definitely solved by experiments with fruit trees; perhaps some of them never will be. Data which can be analyzed quantitatively are not very abundant in horticultural literature in spite of the importance of the question and of the large number of experiments which have been made.

FREQUENCY DISTRIBUTIONS OF YIELDS

As a first step in studying the yield of fruit the homogeneity of the population was investigated, yields being used as a criterion. The results were assembled in classes differing by 40 pounds each and the frequencies plotted as shown in figure 4.

Most of the yields fell between 100 and 250 pounds. The highest yields were in the 400-pound class, and the lowest in the 40-pound class. The curve is asymmetrical because there were more high-yielding than low-yielding trees. A certain amount of asymmetry is

generally found in curves like this, and a comparison of the yields of other years will show that there was generally more asymmetry than in 1922. The greatest numbers of individuals fell into the 160- and 200-pound classes. The actual average yield per tree for 1922 was 194.3 ± 2.8 pounds.

For the purpose of this study the most important result of this arrangement is the evidence that we are dealing with a homogeneous group of trees. If the curve had more than one well-defined hump we should be suspicious that there was more than one biological group in the population, and it would be difficult to study or analyze the data by the simple mathematical methods to be described in subsequent paragraphs.

An inspection of the curves representing the frequency distribution of yields for successive years brings out some significant facts.

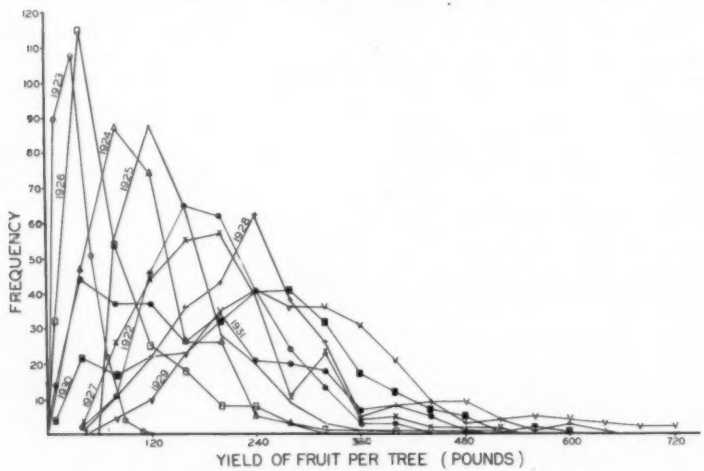


FIGURE 4.—Frequency distributions of yields of all trees, 1922-31.

When the yields were large, as in 1929 and 1930, there was a wider "spread" than in years like 1923 and 1926 when yields were small. It seems logical to conclude that small yields were due to the action of factors which had almost similar effects on all trees and that large yields were due to factors which affected the trees diversely. The skewness of the curves to the right indicates that there was a group of persistently higher yielding trees in the orchard. This group (as will be later explained) consisted in large part of the unpruned trees, but some of the plots receiving pruning frequently fell into this class.

The next step in the study is an investigation of the yields of the plots pruned by the 10 systems listed on page 2.

QUARTILE DISTRIBUTION OF YIELDS

The production of 200 individual trees (omitting the records of the unpruned trees in the control plots), the average of their production

for the 10 years, 1922 to 1931 inclusive, is shown in figure 5. The trees were classified on the basis of the quartile.[4] The 50 trees producing the least fruit were put into the first quartile, and so on. The actual limits are given in the legend of figure 5. This is an excellent means of expressing the actual production of the population under study and also of observing effects of soil conditions.

The right half of the orchard was less productive than the left half. There was a group of 12 trees in block B all of which were in the first quartile, largely because of die back of the tops, for which no definite cause was found. Another group of low-yielding trees in blocks C and D stood on shallow soil. There is a striking place effect in the marginal trees due to freedom from competition of adjoining apricot trees. Three fourths of the trees in the outside rows were in the fourth quartile. This high production cannot be considered a result of pruning treatment, since the rest of a plot was frequently in a lower quartile. The diagram also enables one to place the various plots with respect to each other. The advantage of the Latin-square method of distributing the subplots is apparent. There is no difficulty in seeing that the yields of the trees in plot 1 were relatively low, while those of plot 8, or plot 10, were relatively high, but a more precise measure can be obtained easily by a simple calculation.

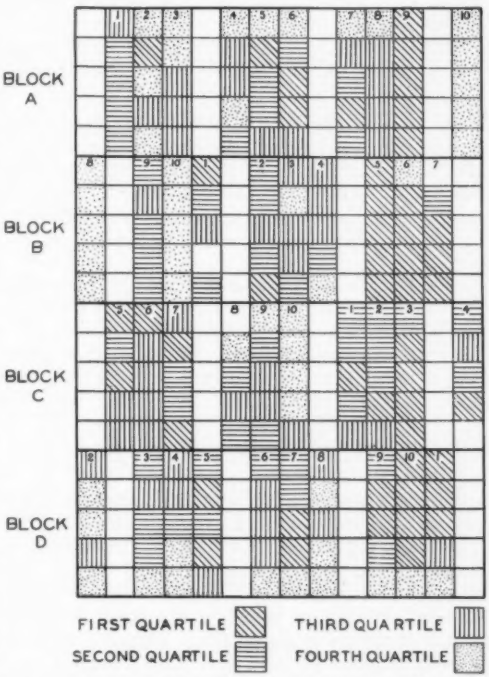


FIGURE 5.—Quartile distribution of average yields of individual trees, exclusive of unpruned trees in control plots. Each quartile contained 50 trees. The ranges were: First quartile, 75 to 132 pounds; second quartile, 133 to 148 pounds; third quartile, 149 to 172 pounds; fourth quartile, 173 to 273 pounds

There are four quartiles. If the yields of trees were distributed by chance there would be an equal number of trees from each of the quartiles in each plot and the mean quartile value of a plot would be—

$$n\frac{(1+2+3+4)}{4n}=2.5$$

[4] In using the term "quartile" to designate a portion of the population, the author is aware of some danger of confusion of terms. Strictly speaking, a quartile is a point so situated on the base of a frequency polygon that one fourth of the individuals lie on one side and three fourths on the other. In this paper as in the preceding one (*4*), [5] the term quartile will be used to designate one fourth of the population.

[5] Reference is made by number (italic) to Literature Cited, p. 29.

A mean quartile value of less than this indicates that the plot contained more low-yielding trees than would have fallen there on the basis of a purely random distribution; if the mean quartile value is greater than 2.5, the plot contains more high-yielding trees than one would find by chance. The greater the difference, the greater the extraneous influence which determined the mean quartile value. This method affords a ready means of judging the effect of various types of pruning on yields in which the yield of each tree finds expression.

The standard deviation expresses the "scatter" of the individual values around the mean. The standard deviation of the numbers 1, 2, 3, 4 is—

$$\sigma = \sqrt{\frac{4^2-1}{12}} = 1.12$$

That means that if there were an equal number of trees from each quartile in each plot, the standard deviation would equal 1.12. If the observed standard deviation of a plot is less than this, we may justifiably infer that the quartile values cluster more closely to the mean than they would in a purely random distribution. The values under discussion are given in table 4.

TABLE 4.—*Mean quartile values of the yields of apricot trees and standard deviations grouped in plots*

Plot no.	M	σ	Plot no.	M	σ	Plot no.	M	σ
1	2.11	0.85	5	1.80	0.93	8	3.42	0.67
2	2.70	1.05	6	2.35	1.11	9	2.00	.95
3	2.05	1.14	7	1.90	.99	10	3.35	1.19
4	2.90	.85						

There were three plots whose trees had mean quartile values well above 2.5, or, as one might say, contained more than their share of high-yielding trees. We may consider the implications of these results. Plot 8 had the highest mean quartile value and a low standard deviation, indicating that the yields tended to cluster more closely about the mean than they would if the distribution were purely random in character. Figure 5 shows that subplot 8 in block B was on the outside row and that the yield of each tree in that subplot was in the fourth quartile. This position doubtless raised the rating of this plot. However, if these 5 trees had been omitted from the calculations there still would have remained 5 trees of the fourth quartile, 7 trees of the third quartile, and 2 trees of the second quartile. Obviously they rated above the average.

Plot 10 also had a high quartile value, but the standard deviation of the mean is slightly larger than that of a purely random distribution. The results indicate that in spite of variability here is a high-yielding plot, though some of its superiority is due, no doubt, to the marginal position of one of the subplots.

Plot 4 is also conspicuous for a high mean quartile value associated with a rather small standard deviation, yet no subplot was located where it had the benefit of the marginal position.

These three plots had a few characters in common which may deserve comment: (1) Vase-shape trees. All these trees were pruned to maintain open centers, with a consequent production of fruit on short laterals arising from the main limbs. (2) Moderate pruning. The amounts of wood removed were smaller in comparison with the size of the trees than on other plots, especially in the early years of the experiment. (3) Winter pruning was not uniformly better than summer and winter pruning. In fact the yields of plots 4 and 8 are directly comparable in this respect. The results support the arguments in favor of winter pruning, but they are not so wholly conclusive on that point as other results yet to be presented.

Plots 2 and 6, which have mean quartile values and standard deviations differing very little from the theoretical, were pruned alike except that plot 2 was pruned in the summer as well as in the winter.

Plots 1, 3, 5, 7, and 9 have mean quartile values considerably less than the theoretical, indicating that something happened which definitely lowered their fruit production. Plots 1, 3, 5, and 7 were all heavily pruned. The types of pruning on plots 1 and 3 corresponded to those on plots 5 and 7 except that the time varied. Plot 9 falls in this group because the trees were damaged by girdling. The standard deviations of the means are significantly less than the theoretical, indicating that some factor other than chance operated to place them where they are found.

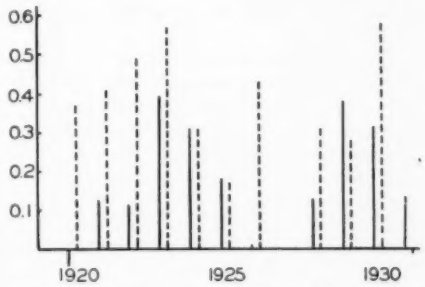


FIGURE 6.—Correlations between weight of wood removed by pruning and yield of fruit. Continuous lines represent values of r for plots 1 to 10; values less than 0.15 are not significant. Broken lines represent values of r for plots 1 to 4.

The results of these simple calculations bring out in brief form the relative superiority of the types of pruning employed so far as the total production of fruit was concerned.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

We may turn now to the correlations between the amount of wood removed, regardless of the type of pruning, and the yield of fruit.

The coefficients of correlation between w (weight of wood removed at pruning) and y (yield of fruit produced in the following summer) are shown both for plots 1 to 10 and for plots 1 to 4, as vertical lines in figure 6. The magnitude of the correlations is in general surprisingly small and irregular. The values of r_{wy} for plots 1 to 10 were very close to zero in 1920 and 1926. We might expect an absence of correlation in 1920, when, on account of immaturity, the trees produced a small crop which averaged only 18.25 pounds per tree, or in 1926, when the trees produced a crop which averaged only 67.50 pounds per tree. We cannot, however, apply this explanation to the low values of the correlations in 1922 or 1928 when large crops of fruit were produced.

The graphs show that the values of r_{wy} for plots 1 to 10 were positive and significant in 5 of the 11 crops recorded. We may with propriety disregard the small crops of 1920 and 1921 and say that there were positive significant correlations 5 times in 9 years. This is better, but it is not so significant that it can have great value in forecasting the probable results of pruning if yield of fruit is the sole criterion. There are nevertheless some important observations to be made on the coefficients under discussion. It will be noticed that all the significant coefficients are positive. This effectively disposes of any argument that pruning, severe as it was, decreased yield of fruit. Severe pruning means that in some cases the prunings from a single tree weighed as much as 150 pounds. On the other hand, we may safely say that, in 5 of the 9 years, the trees which bore the heaviest crops of fruit were those from which more wood was removed.

Figure 6 also contains vertical broken lines which represent graphically the coefficients of correlation, r_{wy}, for the 4 plots which were pruned in midsummer as well as in winter. In 9 of the 11 years the coefficients were positive and more than 3 times their probable errors. Hence the coefficients of these plots indicate a stronger positive correlation between pruning and yield than those of the entire 10 plots.

The question arises, "Did the summer pruning actually affect the physiology of the tree in a way that harmonized the trends in pruning and yield, i.e., heavier pruning and heavier yield, or is this the result of using a more homogeneous population?" The answer involves a knowledge of the variability of the weight of prunings and the yield. The products of the standard deviations are an excellent measure of the dispersion of the values of w and y about their mean values, and are easily obtained from the computations of the various values of r_{wy} for the 11 years. The mean value of $\sigma w \ \sigma y$ for plots 1 to 4 is 766 and for plots 1 to 10 is 1,432. The difference between the 2 products is evidence that the first-named plots of trees were more homogeneous than the last-named plots and accounts for the higher correlations in the case of plots 1 to 4 inclusive. The fact that the summer- and winter-pruned plots produced somewhat less fruit than plots 5 to 10 inclusive suggests that any leveling factor at work tended to reduce the yield of the higher producing trees to average or subaverage amounts.

Further considerations have shown that the inclusion of data from plot 10, the "long-pruned" plot, was responsible for the smaller coefficients of correlation of plots 1 to 10 inclusive. For example, the data was taken for plots 1 to 9 for 1926 (a year in which the correlation was approximately zero). For these plots a value of $r_{wy}=0.335\pm0.045$ was obtained in place of 0.009 ± 0.048. The value of r_{wy} for plot 10 was 0.005 ± 0.132 for the same year. From this it appears that the inclusion of the records of plot 10 had much to do with the lack of correlation indicated above. The discrepancy is due to the fact that the difference between plot 10 and the others was not solely in the amount but in the type of wood removed. The pruning operations on plots 1 to 9 removed the terminal portions of practically all the shoots which had grown in the preceding summer, while on trees of plot 10 they consisted essentially of thinning the

shoots without removing the terminal portions. If a branch was shortened by the removal of the terminal portion, it was cut immediately above a lateral branch, avoiding what is ordinarily designated as "heading back."

The influence of the growth of the tree on the values of r_{wy} may be considered next. It needs no lengthy discussion to show that the trees which produced the most new growth had more material removed when pruned, perhaps not relatively, but absolutely. Therefore it is advisable to determine $r_{wy.t}$, the coefficient of partial correlation. The values are shown in figure 7. The coefficients here shown make it more than ever apparent that there is no real correlation between the severity (within limits) of pruning and the yield of fruit. Although there was a small negative correlation between the pruning and yield in 3 of the years, there were 4 years in which the correlations were positive and equally strong. This emphasizes the necessity of experiments extending over a period of several years. If, for example, one had experimented with the trees only when they were young and had seen the negative correlations obtained in 1921 and 1922 he might have concluded that pruning is antagonistic to yield, a relationship which was not seen again until 1926. There were only 4 years in which there were good positive correlations in the plots which were pruned in the summer as well as in the winter.

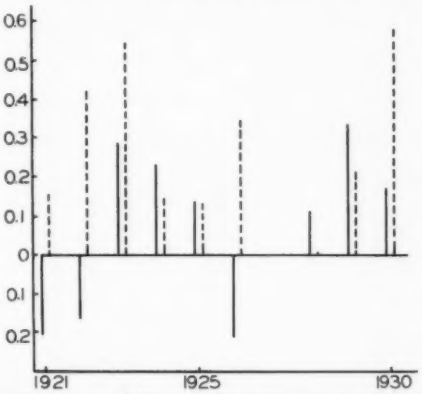


FIGURE 7.—Coefficients of partial correlation. Continuous lines represent values of $r_{wy.t}$ for trees in plots 1 to 10, broken lines those for plots 1 to 4.

The coefficients of correlation between weight of wood removed and the yield of the following year were also of doubtful significance. The values of $r_{w_x y_{x+1}}$ were determined for 10 years. Eight of the coefficients were positive; 2 were negative in sign. Their mean value was 0.203. In 4 of the 10 years the correlation coefficient was larger than 0.3 and positive; in 1 year it was less than -0.3. It is evident that there was no such strong correlation as in the case of $r_{t_x y_{x+1}}$, to be discussed later.

Summarizing the discussion on this problem, it may be said that there is no evidence that the pruning of these 10 plots of apricot trees in the dormant period had any consistent effect upon the amount of fruit the trees produced in the following summer. If the study is confined to the 4 plots which were pruned in the summer immediately after harvest, as well as in the winter, a positive relationship will be found between the amount of wood removed and the yield of fruit in only 5 of the 9 years for which data are available. The unpruned trees produced more than those pruned by any methods whatsoever, but the quality was so poor that the fruit had no commercial value.

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND NUMBER OF FRUITS PER POUND

The correlation between weight of wood removed and number of fruits per pound is a matter of primary importance not only from a biological but from an economic standpoint. Experience has shown that under the climatic and soil conditions in southern California, pruning is an important factor in producing fruit of the required quality.

The size of fruit varied from year to year, but the relative sizes on the 10 plots were in a general way rather constant (table 5). That is to say, the smallest fruits were produced on the unpruned trees and the largest on trees regularly pruned during the winter. Trees pruned in the summer and winter generally produced fruit a little smaller than those pruned only in the winter. There were variations in individual trees which are not readily shown in the averages in table 5, but are reflected in the coefficients of correlation.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

Correlations between the weight of wood removed during the dormant period and the average number of fruits per pound on each particular tree in the following summer were determined for the plots. After 1928 the data from plots 9 and 10 were omitted because the trees on plot 9 were injured by girdling and the type of pruning employed on plot 10 produced results unlike those on the other plots. The coefficients of correlation r_{wn} are represented graphically in figure 8. In the case of plots 1 to 10, values less than 0.125 are not significant, and in the case of plots 1 to 4, 0.200 is the smallest value having significance, assuming that r must exceed three times the probable error. The coefficients were significant in 7 of the 9 years for which there were records, and all the significant coefficients were negative.

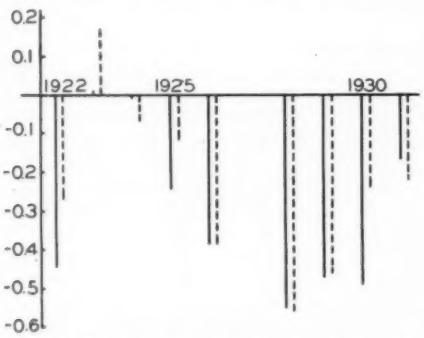


FIGURE 8.—Correlations between weight of wood removed and average number of fruits per pound. Values less than 0.125 are not significant. Continuous lines represent values of r_{wn} for plots 1 to 10; broken lines those for plots 1 to 4.

The mean value of r_{wn} was -0.299. The root-mean-square deviation of the nine computed values of r from the mean is 0.200, indicating a rather wide dispersion of the coefficients during the 10-year period. We should predict from these results that the more severe types of pruning would tend to produce fruit of more desirable sizes, especially as the trees grow older, though there might be years in which some other factor (climate or cultural practices) would interfere with the expression of the true relationship. A line of trend is difficult to establish because of the great variability in the first 4 years in the values of r. Fitting a straight line to the data gives the equation $y = -0.140 - 0.029x$, but extrapolation of this line is impossible because of its slope.

TABLE 5.—*Average number of fruits per pound on trees pruned at different seasons and by various methods, 1922–31*

Plot no.	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931
TREES PRUNED SUMMER AND WINTER										
1	15.3±0.4	9.3±0.2	10.3±0.2	12.5±0.2	8.4±0.4	12.7±0.2	12.0±0.4	13.9±0.4	14.6±0.4	14.4±0.7
2	17.7± .4	8.3± .2	10.4± .2	12.3± .2	8.5± .4	13.1± .2	12.1± .2	14.7± .4	15.1± .4	13.7± .6
3	15.0± .6	9.0± .2	9.8± .1	13.4± .2	8.0± .2	13.3± .2	11.4± .2	14.6± .4	13.0± .3	16.6± .7
4	18.0± .5	8.9± .2	10.0± .1	14.1± .2	7.7± .3	13.0± .3	10.8± .2	13.7± .4	13.9± .3	16.2± .6
TREES PRUNED IN WINTER										
5	15.0±0.5	8.8±0.2	9.4±0.2	12.5±0.1	8.0±0.4	12.8±0.2	10.9±0.3	13.9±0.4	13.0±0.3	13.4±0.6
6	15.9± .4	8.9± .2	9.5± .2	12.6± .2	8.3± .5	12.3± .2	10.2± .2	12.6± .4	12.7± .3	12.9± .6
7	13.4± .5	8.9± .2	9.1± .2	12.2± .2	8.1± .3	11.9± .2	9.7± .3	13.0± .5	11.6± .2	13.7± .4
8	17.4± .8	7.9± .2	9.7± .1	13.0± .3	7.8± .5	13.3± .2	10.3± .2	13.0± .3	11.9± .3	13.4± .3
TREES GIRDLED										
9	17.6±0.5	9.1±0.2	10.0±0.1	13.1±0.2	8.0±0.2	13.3±0.2	10.6±0.2	15.7±0.4	12.2±0.3	16.1±0.4
TREES NOT HEADED BACK, MODERATELY PRUNED										
10	28.0±0.7	10.9±0.2	10.6±0.1	15.5±0.2	10.1±0.3	19.4±0.3	12.6±0.2	19.6±0.5	14.3±0.2	21.7±1.0
CONTROL TREES										
A [1], B [2], C [3], D [4]	32.7±0.5	12.4±0.2	13.3±0.2	19.3±0.2	10.1±0.3	24.7±0.5	13.4±0.3	21.0±0.7	16.4±0.5	20.3±0.6

The coefficients representing the correlation r_{wn} for the four summer-pruned plots were also significant and negative in 6 of the 9 years. In 5 years the coefficients of the summer-pruned plots were approximately the same in magnitude as those of the nine plots. Two of the three years in which there was no significant correlation between pruning and size of fruit were years in which the crop was so light that the size of fruit was not affected by pruning.

The outcome of these calculations makes it clear that the size of fruit was favorably influenced by the pruning. From time to time there was a year in which there was little if any correlation; yet in the long run there was larger fruit where the trees had been more severely pruned.

The number of fruits per pound was possibly dependent upon the yield as well as on the pruning, although, as previously stated, the severity of the pruning had no very apparent relation to yields. Nevertheless $r_{wn \cdot y}$, the coefficient of partial correlation has been computed, which indicates the degree of correlation which would have existed between the weight of wood removed and the number of fruits per pound if all trees had yielded the same amount of fruit in that particular year (table 6).

TABLE 6.—*Coefficients of partial correlation for* $r_{wn \cdot y}$

Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4
1922	−0.561	−0.390	1925	−0.448	−0.282	1929	−0.413	−0.460
1923	−.273	−.123	1926	−.387	−.619	1930	−.535	−.408
1924	−.081	−.142	1928 *	−.590	−.551	1931	−.338	−.248

* The records of plots 9 and 10 were omitted from the calculation.

The results presented in table 6 are less variable than the gross coefficients and speak still more strongly, therefore, in favor of pruning as a means of improving the quality of the apricot crop, since only in 1923 and 1924 were these coefficients without significance.

SOME EFFECTS OF REMOVING THE APICAL PORTION OF SHOOTS

The dominant influence of the apical region in controlling the growth of subapical buds has been described in a previous paper (*6*). The results of this work have established the existence of an important physiological action of the apical meristematic region. Horticulturists have long followed the practice of "pinching" or heading back shoots to encourage the formation of laterals where they will be most advantageously placed. Casual observation of the habit of growth of an apricot tree shows that the apical dominance is very strong. The apex of the shoot dominates the growth, not only on the new wood, but on wood produced several years previously. If undisturbed, the apical region has a strong inhibiting action on the development of the fruiting laterals on the older wood. At the same time, the excessive number of spurs on the young wood bear so many fruits that few of them reach a size having a commercial value.

The effects of heading back were clearly shown in comparing the average number of fruits per pound on the plots pruned by different methods. The largest fruits were obtained from plots 5, 6, 7, and 8;

the fruits produced on plot 10 (not headed back) were small and in some years unmarketable. Hence a system of pruning in which the apical region of new wood is continually removed is of value under southern California conditions.

CORRELATION BETWEEN YIELD AND NUMBER OF FRUITS PER POUND

We may next inquire whether the size of the crop had any influence upon the size of the fruit. The results of such an inquiry might be expected to answer the question "Does a heavy yield of fruit mean that the number of fruits per pound will be large?"

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

The values of r_{yn} the coefficient of correlation for these two variables, yield and number of fruit, were determined for each year from 1922 to 1931 inclusive and are represented graphically in figure 9.

The values of the coefficients or correlation for the plots (1 to 10) which were pruned in the winter were largest in 1922, 1925, and 1931, i.e., in years when moderately heavy yields were produced. To be significant the coefficients should have values of 0.15 or greater. In 5 of the 10 years under observation the coefficients are less than three times their probable errors and may therefore be disregarded. The coefficients range from -0.094 ± 0.053 to $+0.530 \pm 0.036$. Their average value is 0.194, which is doubtfully above the threshold of reliability.

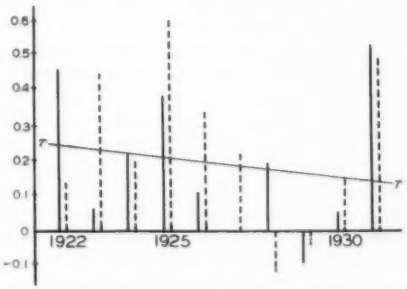


FIGURE 9.—Correlations between yield and number of fruits per pound. Values less than 0.15 are not significant. Continuous lines represent values of r_{yn} for plots 1 to 10; broken lines those for plots 1 to 4; T T, line of trend.

The coefficients indicate that in half the years larger crops were correlated with more fruits to the pound, i.e., smaller fruits, but through the 10-year period there is no strong evidence that small crops meant large fruits.

The fruit on these trees was thinned in 1924, 1925, 1927, and 1928. In 3 of the 5 years in which the positive correlations were significant the crops had been thinned. Thinning consisted of removing all but 1 from the clusters of 2 to 6 fruits in late April before the contents of the seeds had lost their gelatinous consistency. This operation is in harmony with good horticultural practice in the locality under consideration. Theoretically the thinning would reduce the value of the coefficient of correlation because trees bearing a large crop would have larger fruits as a result of the thinning. There may have been some reduction of this kind, but there is little weight for such an assumption if one notes the values of the coefficients for 1929 and 1930, when large crops were produced. It was only in 1927 that thinning coincided with a lack of significant correlation.

These coefficients make it obvious that there is no such fixed relationship between yield and size as might have been forecast since

only about half the correlations are significant. The large crop of 1929 had a small minus correlation, but other large crops in 1922 and 1928 were positively correlated with fruit size.

The equation to the line of trend, calculated by the method of least squares, is

$$y = 0.259 - 0.012x$$

The line of trend indicates that the correlations would tend to diminish with the increasing age of the trees. That is to say that other factors than yield would probably determine the size of fruit. Among these factors we can recognize (1) competition between the root systems as the trees reached maturity, (2) light conditions, which are often unfavorable to the fruit wood in the interior of large trees in spite of the system of pruning employed, and (3) pruning. It is probable therefore that size of fruit and size of crop will not be definitely correlated more than half the time.

The coefficients of correlation for the plots which received summer and winter pruning are in five of the cases larger than the coefficients for plots 1 to 10, but in a general way they are in harmony with those of the entire 10 plots. The most pronounced disagreements were in 1922, 1927, and 1928.

Since it has been shown that the severity of the pruning (weight of wood removed) affected both the number of fruits per pound, and, to a smaller degree, the yield, it was advisable to determine the coefficients of partial correlation $r_{yn \cdot w}$. These coefficients (table 7) are somewhat larger than those of gross correlation. Six of the nine in the table are large enough to have statistical significance. These figures do not, however, contribute definite support to the general assumption that there is much correlation between yield and size of fruit. This absence of correlation between fruit size and magnitude of yield has also been observed in orange yields (7). The general problem merits further careful study.

TABLE 7.—*Coefficients of partial correlation for* $r_{yn \cdot w}$, *1922–31*

Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4
1922	0.465	0.322	1925	0.450	0.634	1929	−0.035	0.105
1923	.067	.432	1926	.124	.600	1930	.252	.367
1924	.237	.232	1928	.320	.064	1931	.773	.502

CORRELATION BETWEEN YIELD AND THE GROWTH OF THE TREE

Correlation between yield and the growth of the tree is another problem of biological importance because it bears directly upon the question as to whether the process of fruit production is more or less opposed to vegetative growth. An investigation of the data in hand ought to afford some information on this question. By many investigators the phenomenon of bearing in alternate years in certain trees is generally referred to the competition between fruit production and vegetative growth. Where the supply of material is limited the reproductive processes of the organism usually attract the major portion of the material acquired.

COEFFICIENTS OF GROSS CORRELATION BETWEEN THE GROWTH AND YIELDS OF TREES IN THE SAME YEAR

Figure 10 shows the values of the coefficients of correlation, r_{iy} where i is the increase in the area of the cross section for any year and y is the yield of fruit for the same year. The coefficients are not large enough to have significance except in 1927, indicating that over the period of observations there was no well-marked correlation between yields and the growth of the tree in the corresponding year.

The trend of the coefficients was calculated from the observed values by the method of least squares. The equation to the line of trend is

$$y = 0.204 - 0.0049x$$

The mean observed value of the coefficients is 0.194, but the root-mean-square deviation from the values calculated by the equation to the line of trend is 0.194. Since the observed values are so widely dispersed the line of trend as calculated has little significance.

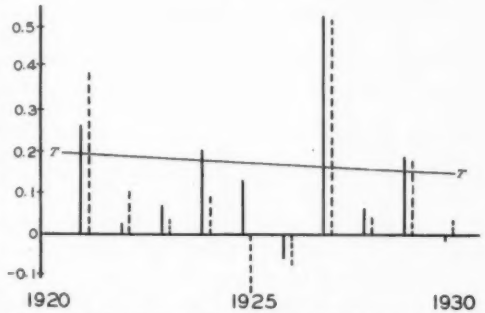


FIGURE 10.—Correlations between increment in the area of cross section and the yield of fruit in the same year. Continuous lines represent values of $r_{i_x y_x}$ for plots 1 to 10; broken lines those for plots 1 to 4; *T T*, line of trend.

If the processes of reproduction and wood growth were mutually opposed we should obtain coefficients which were negative in sign and significant in magnitude. But clearly there is no evidence of that condition, nor is there any reliable indication in these data that the two processes are positively correlated. There is, as is well known, an opportunity for the apricot tree to grow for 4 months after the crop of fruit has been harvested (*5*).

COEFFICIENTS OF GROSS CORRELATION BETWEEN THE GROWTH AND YIELDS OF TREES IN THE SUCCEEDING YEAR

Another correlation of considerable importance has been determined, namely, the correlation between yields of the trees and their increments of growth in the preceding year. These computations were based on the assumption that the condition of the tree at the end of the growing season (indicated by the increase in the area of the cross section during the whole season) has more influence upon the yield of the tree than the amount of growth made from the blossoming period to time of harvest. Accordingly a series of computations of the values of $r_{i_x y_{x+1}}$ were made, which are represented graphically in figure 11. There is a great difference between the values shown in figures 10 and 11. The coefficients expressing the values of $r_{i_x y_{x+1}}$ are positive and, with one exception, statistically significant. This is good evidence that the yields are more intimately correlated with the growth (measured by area of cross section) of the preceding year than with the growth of the same year.

In both series of computations there is a sag in the value of the coefficients in 1925 and 1926, followed by a conspicuous rise in 1927.

This sag is not readily explained. The average production in 1925, 1926, and 1927 was 148, 73, and 195 pounds of fruit per tree, respectively. The prunings (weight of wood removed) in 1925 and 1926 were 81 and 65 pounds, respectively. The record for 1927 was lost, but it was probably near 60 pounds. The difference, therefore, between the amount of wood removed in 1926 and 1927 was less than between the amounts removed in 1925 and 1926. There is little evidence from these figures that the sag in the values of these coefficients was due to radical differences in the amounts of wood removed by pruning. It is more probable that this sag was related to an infestation of black scale on certain trees in the center of the orchard. The insects appeared in 1924 and increased until exterminated by a period of high temperature which rose to 119° F. on July 17, 1925. The growth increments of the trunks of numerous trees in block B (fi . 1) considerably below the average of the field in 1926, whereas in other years they were generally above the average. It is possible that the retardation of growth by this infestation accounts for the lower correlations noted.

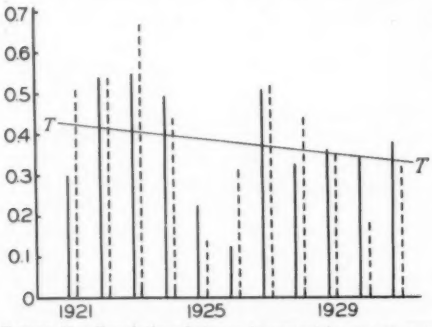


FIGURE 11.—Correlations between increment in area or cross section and yield of fruit in succeeding year. Continuous lines represent values of $r_{t_x y_{x+1}}$ for plots 1 to 10; broken lines those for plots 1 to 4; *T T*, line of trend.

The equation to the line of trend of the coefficients expressing $r_{t_x y_{x+1}}$ is

$$y = 0.434 - 0.0094x$$

and the mean observed value of the coefficients is 0.377. The deviation of the observed from calculated values is only 0.122, indicating that the calculated values are fairly representative. This indicates that the initially high degree of correlation between growth of the previous year and yield observed in young apricot trees will decline slowly with time. The trees will grow more slowly as they approach maturity, and the yields will be less highly correlated with the annual growth of each antecedent year. As a matter of fact the horticulturist knows that the yields of mature trees are more acutely affected by the incidence of insect infestation or fungous diseases than by other factors which affect directly the physiology of the apricot trees.

It is unfortunate that so many of the published accounts of horticultural experiments, especially those concerned with pruning, have been conducted on young trees. More reliable information may be had from trees whose root and branch systems fill approximately all the space at their disposal, i.e., when the competition for the components of the soil and for light becomes really acute.

The correlation $r_{t_x y_{x+1}}$ for the trees in plots 1 to 4 (summer-pruned) is so nearly the same that no additional discussion is necessary.

CORRELATION BETWEEN GROWTH OF THE TREE AND NUMBER OF FRUITS PER POUND

COEFFICIENTS OF GROSS CORRELATION

There is a fairly strong correlation between the increment in the area of the cross section and the average number of fruits per pound. The value of r_{in} are shown graphically in figure 12. In 6 of the 9 years for which there are data a fair negative correlation existed. This is as one might expect. The trees that were growing most vigorously were also producing large fruits. In other words, vigorous growth of the vegetative phases of the tree was coupled with vigorous growth of the fruiting organs.

The mean value of r_{in} is -0.272. The trend is represented by a line calculated from the equation,

$$y = -0.260 - 0.0024x$$

The root-mean-square deviation of the observed from the calculated values of y is 0.132, which implies that the line of trend may be accepted with a fair amount of confidence.

On comparing the results of the computations dealing with the relation between the yield and number, and the relation between the yield and growth of the tree, the reader will probably be impressed by the evidence that fruit production in the apricot tree is more nearly comparable to vegetative than to reproductive functions. At all events there is no significant evidence to show that the types of pruning employed restricted the yield of these trees. On the contrary it seems that the growth of the tree was strongly correlated with the yield in the succeeding year, indicating that the two functions go together. This is not surprising when one considers that the yield of fruit depends largely on the thickness of the flesh surrounding the pit, the mesocarp. The mass of the pit is not as variable as that of the fruit, but the thickness of the flesh depends so largely upon the vegetative functions of the tree that it is logical to expect that it should vary with other functions in that category. If it were otherwise one would not expect to see the beneficial results on fruit production obtained from the improvements in the technic of orchard care which are usually obtained in good horticultural practice.

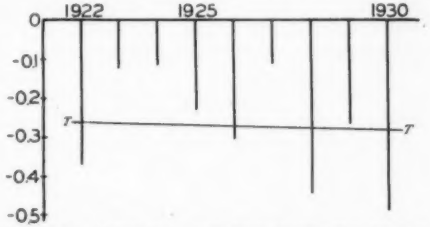


FIGURE 12.—Values of r_{in}, coefficients of correlation between increments in area of cross section and number of fruits per pound; *T T*, line of trend.

The small average correlation between the severity of pruning and the yield of fruit, either of the same or of the succeeding year, has been rather fully discussed. It was shown that part of the discrepancy arose from the fact that the system of light pruning applied to plot 10 produced a reaction differing specifically from that obtained from the system of heading-back applied to other trees. This important discovery lends support to the conclusion, reached independently from the other results, that this type of light pruning had little to do

with the magnitude of the yields, and that it is not likely to be successful in a locality where apricot trees are prone to produce heavy crops of fruit. The correlation between the severity of the pruning and the number of fruits per pound is more significant and confirms the southern California practice of heading back apricot trees to produce fruit of superior quality.

INTERANNUAL CORRELATIONS

GROWTH OF TREES

One of the problems of paramount interest to the orchardist is the development of his trees. He is concerned not only about the growth of the trees, but about the uniformity of their growth. It is well-known that regularity in growth is generally associated with regularity in fruit production. If the type of pruning or any other cultural operation seriously altered the uniformity of growth of the trees it would sooner or later impair the vitality and productiveness of the trees.

Accordingly some computations on the coefficients of correlation between the size of the trees when measured annually before the growing season started are here presented and discussed. These interannual correlations may be expected to show whether there was any well-defined tendency for the trees of selected plots to grow irregularly and whether the trees which grew most in a given year grew least in succeeding years.

The labor of computing interannual correlations for all plots would be very great; consequently three plots were selected which represent rather distinct types of pruning and the computations confined to them. Plot [1], a control plot, represents the unpruned or nearly unpruned tree. It is of interest because it was well-cultivated, sprayed for pests, and well cared for in all ways except pruning. The trees comprising plot [1] lie on the extreme left side of the orchard, while those in the two other plots were distributed over a larger area (fig. 1). This would vitiate the conclusions if yields or sizes were being compared with those of other plots. However, the yields or sizes of the same trees in different years are being compared, and hence the method of computation is justifiable. Plot 3 was pruned severely. Part of the pruning was done in the summer, but most of the pruning, as already stated, was done during the dormant season. Plot 10 was pruned, but not severely, by thinning branches in contrast to heading-back new growth.

The coefficients of correlation for the area of the cross section of the trees are given in table 8. (The measurements for 1927 unfortunately were lost.) The coefficients are remarkably large, and all are positive. Along the diagonal lines of the table the coefficients indicate that there was almost a 1:1 correlation between the sizes in consecutive years. The coefficients for the correlation of any year with a series of consecutive years show some tendency to form a descending series, but the lowest values in the table are generally larger than those obtained in biometrical work. The coefficients of correlation for plots [1] and 3 between 1930 and preceding years were slightly less than others, though they indicate a strong positive correlation. A satisfactory explanation cannot be given for this depression, nor for the absence of a similar depression in the interannual correlations of plot 10.

TABLE 8.—*Interannual correlations of the area of the cross section of control plot [1], plot 3, and plot 10*

CONTROL PLOT [1]

Year	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0.970±0.038	0.886±0.072	0.707±0.109	0.758±0.101		0.738±0.104	0.665±0.116	0.612±0.122	0.550±0.062
1923		.926± .058	.931± .036	.865± .077		.859± .066	.783± .066	.741± .103	.736± .104
1924			.689± .112	.936± .054		.916± .062	.872± .075	.850± .081	.822± .121
1925				.974± .035		.946± .049	.920± .060	.859± .079	.893± .069
1926						.983± .028	.976± .034	.698± .112	.958± .044
1928							.984± .027	.976± .034	.967± .039
1929								.994± .017	.964± .043
1930									.980± .023

PLOT 3

1922	0.976±0.033	0.977±0.032	0.948±0.048	0.915±0.061		0.899±0.066	0.868±0.075	0.728±0.103	0.836±0.083
1923		.994± .017	.962± .028	.955± .045		.936± .053	.916± .061	.788± .093	.892± .068
1924			.989± .022	.972± .035		.966± .039	.943± .050	.795± .093	.921± .059
1925				.991± .020		.981± .029	.966± .039	.818± .087	.950± .047
1926						.984± .027	.974± .034	.823± .086	.960± .042
1928							.992± .019	.845± .081	.979± .031
1929								.841± .082	.980± .030
1930									.826± .085

PLOT 10

1922	0.994±0.017	0.989±0.022	0.914±0.061	0.968±0.038		0.961±0.042	0.943±0.050	0.945±0.049	0.915±0.061
1923		.995± .015	.969± .022	.984± .027		.964± .054	.957± .044	.954± .045	.927± .057
1924			.999± .007	.990± .015		.969± .025	.974± .031	.972± .035	.950± .047
1925				.995± .018		.984± .027	.979± .031	.879± .037	.962± .041
1926						.988± .023	.984± .027	.883± .029	.966± .039
1928							.988± .023	.969± .022	.977± .032
1929								.996± .013	.983± .018
1930									.994± .017

YIELDS

The statistical reliability of the records of fruit-tree yields based on one season's performance is a matter of considerable importance in the study of horticultural problems. When one wishes to lay out experiments he wishes to know whether the yields of an orchard in the year or two immediately preceding the installation of the experiment are significant indices of what the orchard will yield during the term of the contemplated experiment.

Since the labor and expense of obtaining records of the yield of orchard trees are often very great, it is desirable to know whether the record of one or more seasons may reasonably fulfill the requirements of the experimenter. It has been rather implicitly assumed that the average yield for several years is rather more reliable than the record of a single year. The commercial grower is equally interested in knowing whether there is such a close correlation between the crops of successive years that he can estimate with a fair chance of success what any crop yield will be. If it were possible to do this he could effect economies in pruning, spraying, application of irrigation water, fertilizers, and other orchard operations.

The successive yields of the three plots just discussed in the preceding paragraphs were studied in some detail to obtain possible answers to the foregoing questions. The means and coefficients of variability for the 10 years 1922 to 1931 have been assembled in table 9.

TABLE 9.—*The yield and variability of yield of apricot trees through a period of 10 years, 1922–31*

Year	Plot [1] (unpruned)		Plot 3 (severely pruned)		Plot 10 (lightly pruned)	
	Mean yield per tree	Coefficient of variability	Mean yield per tree	Coefficient of variability	Mean yield per tree	Coefficient of variability
	Pounds	*Percent*	*Pounds*	*Percent*	*Pounds*	*Percent*
1922	255.9± 5.9	15.1±1.8	141.5± 3.7	18.6± 2.1	237.5± 8.6	24.0±2.7
1923	21.9± 1.5	42.9±5.4	45.0± 3.7	54.8± 7.7	21.8± 1.2	35.2±4.2
1924	195.1± 6.1	20.3±2.3	70.0± 3.4	31.7± 3.7	81.5± 5.4	44.0±5.5
1925	236.3±11.3	29.6±3.5	128.5± 5.0	25.2± 2.8	139.5± 5.3	25.3±2.9
1926	155.1±12.1	50.2±6.7	67.5± 8.6	85.1±14.2	56.5± 5.4	63.5±8.4
1927	251.4±12.1	31.1±3.7	178.5± 8.3	31.0± 3.3	160.5±12.5	51.8±5.5
1928	405.5±19.4	30.7±3.7	226.5± 6.5	18.9± 2.0	262.0±12.4	31.3±3.3
1929	413.4±16.8	26.3±3.1	288.0±12.1	27.9± 3.4	403.0±22.9	37.6±5.2
1930	310.5±22.8	47.4±6.2	237.0±14.6	40.8±10.3	269.0±18.1	44.7±6.7
1931	234.7±17.0	46.7±6.1	190.0±13.6	47.6± 7.4	232.0±22.2	63.5±8.4

The large crop of 1922 was followed by a very small crop, but in successive years the mean yields gradually increased until plots [1] and 3 produced about as much in 1927 as they had in 1922. Plot 10 did not equal its 1922 crop until 1928. Each of the three plots produced its maximum yield in 1929.

The coefficients of variability are far from uniform. They are large for 1923 and 1926, when the yields were small, but are also large for 1930 and 1931, when the yields were above the average for the period. It is not surprising that the variability was large when the yields were small because a difference of a few pounds of fruit would then greatly increase the dispersion of the frequency distributions, but the persistence of the variability indicates that

there was a factor operating to cause unequal yields in both cases. In fact a distinct trend in each of these plots toward increased variability may readily be recognized during the whole 10-year period. Apparently the inevitable inequalities in soil conditions influenced the yields of the trees more strongly as the extending root systems competed with each other for water and soil components. Plot 3 (severely pruned) was less variable than one or both of the other plots, except in 1923 and 1926. The high variability in 1931 may be attributed partially to the frost injury already mentioned.

Batchelor and Reed (*2*) showed that the variability of tree yields was not significantly decreased by combining the records of two or more years. Parker and Batchelor (*3*) found that the annual gross variability in the yields of orange trees was of approximately the same order, with the possible exception of the first crop. The correlations of yields of individual trees for consecutive years were positive and undoubtedly significant, although the values of the correlation coefficients diminished as time went on.

In contrast with the data on size of trees the yields were more variable at the end of the experiment than the size of trees.

Table 10 contains the coefficients of interannual correlation between the yields of apricot trees in the three plots. There is a somewhat surprising lack of correlation of yields in contrast with the high correlations of area of cross section just discussed.

The diagonal row of coefficients in table 10 shows that there was a significant correlation between yields of trees in control plot [1] in consecutive years only 4 times in 9 years and that each of the 4 significant coefficients had a positive sign. This means that in four cases the trees which produced heavy crops of fruit in a year like 1922 or 1929 tended to produce heavy crops likewise in the year following. However, in 5 out of 9 cases the coefficients do not indicate any significant correlations between the crops of 2 consecutive years.

When we examine the horizontal rows of coefficients we see that the crop of 1922 on control plot [1] was significantly correlated with a subsequent crop 6 out of 9 times. The crop of 1923 was significantly correlated 4 out of 8 times with succeeding crops, and so on. It is very manifest that there was no regular decrease in the values of coefficients similar to what other investigators (*2*, *3*) have found in the growth of annual plants or in the yields of citrus fruits.

The diagonal row of figures in table 10 for plot 3 showed significant correlations between the yields of fruit of the trees in consecutive years only 3 times in 9 years, viz, 1925–26, 1927–28, and 1928–29. The first of these coefficients is negative; the other two are positive. Reference to table 11 shows that the yield in 1925 was moderately large, almost twice as great as that for 1926, with which it had a negative correlation. Evidently the trees in plot 3, which produced more than an average crop in 1925, tended to produce less than an average crop in 1926. The yield in 1927 was fairly large (the largest up to that time), and it was followed by yields increasingly greater in 1928 and 1929. The yield in 1927 was positively correlated with the yield in 1928, and the yield of 1928 was about equally correlated with that of 1929. These relations contrast strongly with those implied in the coefficient of correlation between the 1925 and 1926 yields, indicating that the trees which yielded more than average crops in 1927 and in 1928 tended again to produce more than average crops in the year immediately following.

TABLE 10.—*Interannual correlations of yields for control plot [1], plot 3, and plot 10, 1922–30*

CONTROL PLOT [1]

	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0.548±0.129	0.748±0.103	0.495±0.134	0.379±0.143	−0.059±0.154	0.584±0.126	0.761±0.100	0.449±0.138	0.315±0.147
1923		.288± .148	.615± .122	.299± .148	.128± .153	.644± .118	.567± .127	.540± .130	−.012± .155
1924			.258± .149	.339± .146	.366± .144	.487± .135	.466± .137	.321± .147	.354± .145
1925				.595± .124	−.186± .152	.409± .141	.367± .144	.498± .134	−.200± .152
1926					−.363± .144	.540± .130	.205± .151	.566± .128	−.041± .155
1927						.165± .153	.698± .111	.059± .154	.654± .117
1928							.643± .116	.872± .149	−.029± .151
1929								.473± .133	.311± .143
1930									−.371± .140

PLOT 3

1922	−0.351±0.141	0.259±0.146	0.182±0.148	−0.037±0.151	0.066±0.150	0.500±0.131	−0.048±0.151	0.166±0.149	−0.385±0.139
1923		.390± .139	−.480± .132	−.219± .147	.660± .113	.331± .142	.746± .100	.047± .151	.564± .125
1924			−.165± .149	−.400± .150	.554± .125	.154± .149	.409± .138	−.147± .149	.200± .148
1925				−.491± .131	.094± .150	.400± .139	−.079± .150	−.015± .151	−.448± .135
1926					−.156± .149	.074± .150	−.248± .146	.380± .139	−.220± .147
1927						.768± .097	.877± .072	.302± .148	.614± .119
1928							.635± .116	.593± .121	.217± .147
1929								.226± .146	.750± .100
1930									−.220± .146

PLOT 10

1922	−0.094±0.150	0.789±0.093	0.514±0.129	−0.055±0.151	0.722±0.104	0.551±0.125	0.660±0.113	0.172±0.149	0.278±0.145
1923		−.157± .149	−.056± .151	.257± .146	.225± .147	.242± .146	.273± .145	−.177± .149	.291± .144
1924			.388± .139	−.232± .146	.698± .108	.341± .133	.645± .115	.488± .132	.241± .146
1925				.117± .150	.449± .135	.658± .114	.237± .147	.416± .137	−.119± .150
1926					−.265± .145	−.027± .151	−.110± .150	−.178± .149	.103± .150
1927						.228± .146	.836± .083	.269± .145	.458± .134
1928							.105± .150	.075± .151	−.375± .140
1929								.217± .147	.786± .093
1930									−.119± .150

Inspecting the horizontal rows of coefficients in table 10, one finds no distinct trend of the correlations of plot 3, with 1 exception; the yield of 1922 showed no significant correlation with any succeeding crop. The small crop of 1923 shows significant correlations with 4 later crops, 3 of which are positive and 1 negative. A similar state of affairs is pictured by the majority of the remaining coefficients with two exceptions. The yields for 1927 showed a fair positive correlation with 2 of the 5 preceding years and with 3 of the 4 succeeding years. The 1927 yields were positively correlated with the small crops of 1923 and 1924, as well as with the large crops of 1928 and 1929. Thus, out of 9 years there were 5 in which there were indications that the trees yielding more or less than average crops in 1927 maintained their relative positions in the population. In a somewhat similar way the yield in 1928 shows a significant positive correlation with 3 preceding and 2 succeeding crops. The yields of 1928 are correlated with all the larger crops taken from the plot during the period.

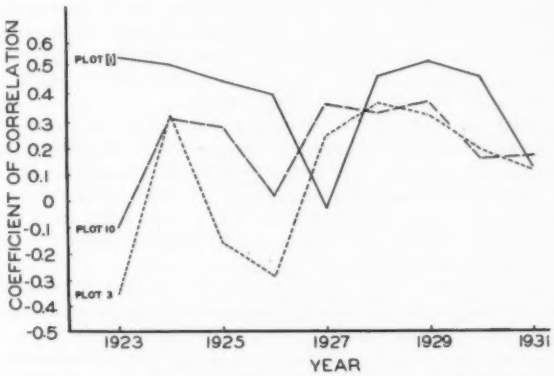


FIGURE 13.—Average interannual correlations of yields of plot [1] (unpruned), plot 3 (severely pruned) and plot 10 (lightly pruned).

The interannual coefficients of correlation between trees in plot 10 may next be examined (table 10). They show somewhat more consistency than those of plot 3, but there is a lack of any well-defined trend. The diagonal row of figures show that there was no definite correlation between the yields of trees in plot 10 in any 2 consecutive years. The coefficients show that the large yield of 1922 was positively correlated with 5 of the 9 succeeding crops, but the small yield of 1923 was not definitely correlated with yields of any of the succeeding years. The yield in 1927 shows somewhat the same degree of relationship to preceding and succeeding years as that of plot 3 showed, viz, a significant correlation with yields in 3 of the 5 preceding and 2 of the 4 succeeding years.

A graphic summary of the interannual correlations of yield is given in figure 13 in the form of 3 graphs representing the average coefficient of correlation for the 3 plots just discussed. The mean for any given year in these graphs is the mean coefficient of correlation between it and the preceding years.

There was no general similarity in the three graphs in 1923, but it should be remembered that each of the points on these graphs for that year is dependent on one coefficient, and has less significance than later values. In each case the values were fairly high in 1924. The average coefficient for the control plot [1] shows a decided minimum in 1927, while the averages for the pruned plots reached minimum values in 1926. The average values in all three cases rose to another maximum in 1929, then declined to 1931. We are not warranted in saying that these small average coefficients are indices to more uniform yields for the years in question. On the contrary the figures in table 9 show that in 1926 and in 1931 the yields were widely dispersed about their means.

The absence of a definite interannual correlation in yields of apricots is rather significant. It indicates that the quantity of fruit produced by a tree is dependent upon factors other than the amount of fruit produced in the preceding year. Whether these determining factors are biological or physical, or a combination of the two, remains to be ascertained. If their nature is biological, such factors as the abundance of pollen-carrying insects, or the prevalence of parasitic insects or of fungi might be investigated. If their nature is physical, it might be temperature, light, water, or that complex assemblage of factors classed as "weather" that was responsible.

The population upon which this study of apricot yields was made was essentially unselected, i.e., heterogeneous, as far as the clon is concerned, although it represented a well-defined type with respect to the species. The variability of the tree yields through the 10-year period was as great as, if not greater than, that commonly found in a random selection of biological material. The variability was likely to be as great in one direction as in another.

In conclusion, the data upon yields of the three plots described show that where annual pruning was practised there was no significant correlation between the yields of successive years and that the correlations between subsequent yields were as large as, or larger than, those of immediately succeeding yields. Significant correlations between yields of unpruned trees were no more numerous than among the plots which were pruned. There is therefore nothing to support the idea that pruning introduced a factor which upset the inherent correlations in yields of consecutive years. It is obviously a matter of chance whether trees which are among the best producers in a given year will produce the best or the poorest crops in the following year. To obtain reliable data on yield it is therefore necessary to collect records for a number of seasons.

SUMMARY

The effects of pruning apricot trees were analyzed by means of the correlation coefficients.

The coefficients of gross correlation show that the amount of wood removed (severity of pruning) and growth of the tree in the succeeding season were positively correlated, but this correlation disappeared when partial correlations involving the size of the tree were made. When the sizes of the tree trunks were computed as percentages of those of the trees on the nearest control plots, the trunks of the trees on 8 plots were found to be smaller and those of 2 larger than the

trunks of the trees on the controls. Therefore it seems that trunk growth was somewhat reduced by the heavier types of pruning.

Frequency distributions of the yields gave unimodal asymmetrical curves.

Six of the plots had mean quartile values lower and four of the plots higher than would have been obtained in a purely random distribution of yields. The highest yields for the 10-year period were obtained on plots whose trees were moderately pruned in winter to a vase type. The standard deviations of the means indicate that some factor other than chance determined the average yield of plots. The coefficients of gross and of partial correlation lend little support to the idea that the quantity of wood removed had significant influence upon the yield. The type of pruning was apparently more important than the severity within the limits of the experiment.

The coefficients of correlation show a strong relationship between the amount of wood removed and the average number of fruits per pound. The more severe types of pruning tended to produce fruit of larger size as the trees grew older, provided there was no marked change in environmental conditions.

The results indicate no strong correlation between the size of fruit and the volume of the crop.

The yield of fruit and the growth of the trees in the same season show no very definite correlation, except in 2 years out of 10, but there is a significant and positive correlation between the yield one year and the growth of the tree in the preceding year. Trees pruned in summer and in winter show practically the same correlations as those pruned only in the winter.

There is a fairly strong negative correlation between the growth of the tree and the number of fruits per pound in 6 of the 9 years for which records were kept. The setting of fruit evidently depends on reproductive and vegetative functions of the apricot tree, while the size of fruit probably depends upon much the same factors as do wood and leaf growth.

Interannual correlations of trunk sizes were computed for a control plot, a severely pruned plot, and a lightly pruned plot. All coefficients were positive and very high in value. Apparently size differences were persistent under the conditions of the experiment.

The coefficients of interannual correlations of yields were computed on the three above-mentioned plots. Their values varied greatly from year to year, showing no general correlation. This indicates that the annual quantity of fruit produced by a tree is dependent upon factors other than the amount of fruit produced in the preceding year.

LITERATURE CITED

(1) Batchelor, L. D., and Reed, H. S.
1918. relation of the variability of yields of fruit trees to the accuracy of field trials. Jour. Agr. Research 12: 245–283, illus.

(2) Harris, J. A., and Reed, H. S.
1921. inter-periodic correlation in the analysis of growth. Biol. Bull. 40: 243–258, illus.

(3) Parker, E. R., and Batchelor, L. D.
1932. variation in the yields of fruit trees in relation to the planning of future experiments. Hilgardia 7: 81–161.

(4) REED, H. S.
1919. GROWTH AND VARIABILITY IN HELIANTHUS. Amer. Jour. Bot.. 6: 252–271.
(5) ———
1921. GROWTH AND SAP CONCENTRATION. Jour. Agr. Research 21: 81–98, illus.
(6) ———
1921. CORRELATION AND GROWTH IN THE BRANCHES OF YOUNG PEAR TREES. Jour. Agr. Research 21: 849–876, illus.
(7) ——— and BARTHOLOMEW, E. T.
1930. THE EFFECTS OF DESICCATING WINDS ON CITRUS TREES. Calif. Agr. Expt. Sta. Bull. 484, 59 pp., illus.

CORRELATED INHERITANCE OF REACTION TO DISEASES AND OF CERTAIN BOTANICAL CHARACTERS IN TRIANGULAR WHEAT CROSSES [1]

By ELMER R. AUSEMUS [2]

Associate agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In order to plan a systematic attack on a plant-breeding problem it is essential to obtain information on the mode of inheritance of certain characters, the reaction of the plant to diseases, and the extent of correlation or linkage, if any, among these various characters. For the purpose of the research presented in this paper triangular crosses were made of Hope, Marquillo, and Supreme, three common spring wheats (*Triticum vulgare* Vill.[3]) to determine (1) the mode of inheritance of reaction to stem rust (*Puccinia graminis* Pers.), bunt (*Tilletia tritici* (Bjerk.) Wint.), and black chaff (*Bacterium translucens undulosum* Smith, Jones, and Reddy), of awn development, and of color of coleoptile; (2) the interrelationship of these characters; and (3) the relation of stomatal behavior to resistance and susceptibility to stem rust. It is recognized, of course, that environment influences many of these characters, making the study of their inheritance very difficult.

REVIEW OF LITERATURE

Considerable work has been done by investigators to determine the inheritance of the two most important characters considered in this paper, namely, reaction to stem rust and reaction to bunt.

REACTION TO STEM RUST

In this paper only those studies having a direct bearing on varietal resistance to stem rust are reviewed.

Melchers and Parker (*32*)[4] found a single-factor difference for rust reaction in a Marquis×Kanred cross. Immunity was dominant when the plants were inoculated at heading time in the greenhouse. Kanred×Marquis crosses have been studied by Aamodt (*1*) and

[1] Received for publication Oct. 11, 1933; issued February 1934. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Minnesota Agricultural Experiment Station. Submitted in partial fulfillment of the requirements for the degree of doctor of philosophy at the University of Minnesota.

[2] The writer wishes to thank Dr. H. K. Hayes, chief of the Division of Agronomy and Plant Genetics, University of Minnesota, for his valuable advice regarding methods of conducting the study and in the interpretation of the results, and Dr. E. C. Stakman, professor of plant pathology, University of Minnesota, for valuable suggestions on those phases of the investigation dealing with disease resistance. Acknowledgment is also made to Dr. Stakman and to Drs. M. N. Levine and R. U. Cotter, of the Bureau of Plant Industry, stationed at University Farm, St. Paul, Minn., for supplying the physiologic forms of stem rust used in the present investigations and for identifying form 36, used in determining the seedling reaction to stem rust in the greenhouse, and to R. H. Bamberg, in charge of the cooperative cereal pathology garden, for the collection of bunt used in testing F_3 lines.

[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L.; but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that name.

[4] Reference is made by number (italic) to Literature Cited, p. 55.

Hayes and Stakman (*26*), who found that one factor may control seedling reaction to several physiologic forms of stem rust. They found also that lines resistant to certain forms of rust in the greenhouse may be susceptible in the field, owing to the presence of other physiologic forms under field conditions or to the difference in reaction of the plants at various stages of growth. Hayes and Aamodt (*24*), working with a Kota × Marquis cross, concluded that the study of F_3 seedlings in the greenhouse was not a satisfactory means of isolating F_3 lines that would exhibit resistance of the Kota type in the field.

Hayes, Stakman, and Aamodt (*27*) crossed Marquis × Iumillo lines, moderately resistant in the mature stage in the field but susceptible in the seedling stage in the greenhouse, with Marquis × Kanred lines, immune in the seedling stage from 11 forms of stem rust. Field resistance was inherited independently of greenhouse reaction to the forms from which Kanred was immune. They concluded that two factors controlled the Marquis × Iumillo type of field resistance in this cross, although minor modifying factors were involved. From these crosses homozygous lines were obtained combining the seedling immunity of the Marquis × Kanred parent with the field resistance of the Marquis × Iumillo parent. There were all combinations of resistance and susceptibility among the hybrids, including homozygous types that contained the factors for resistance of both parents and homozygous types that were susceptible both in the greenhouse and in the field.

Harrington (*20*) reports that there was good agreement between seedling reaction and field reaction of Marquis, Pentad, and Mindum to forms 34 and 21. Tests were made in muslin cages located in the field and the rust forms were checked later in the greenhouse. The greater part of the rust prevalent in 1922 was form 34, and that prevalent in 1923 was form 21. Slight mixtures of other forms also were present.

Aamodt (*2, p. 215*) states: "Immunity or a high type of resistance can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field." He used the same forms in the field and in the greenhouse.

Goulden, Neatby, and Welsh (*18*), working with a cross of H-44 × Marquis, found no relation between seedling reaction to forms 21 and 36 in the greenhouse and the reaction of the mature plant in the field to a collection of forms. They concluded that different genetic factors controlled resistance in the greenhouse and in the field. Two genetic factors were necessary to explain the seedling reaction to form 36 in the greenhouse. Studies made in the field indicated that resistance was controlled by a single pair of factors.

Clark and Ausemus,[5] in a study of Hope crossed with Marquis and Reliance, found in the mature-plant stage immune, resistant, and susceptible strains breeding true and found also segregating types. The results of the study of the F_2 and F_3 generations were explained on the basis of a 2-factor difference. Phenotypic ratios for the immune, resistant, and susceptible groups were approximately 4:11:1. No susceptible plants or strains were obtained in a Hope × Ceres cross, i.e., a cross between immune and resistant parents. These

[5] Clark, J. A., and Ausemus, E. R. Inheritance of immunity from black stem rust, yield, and protein content in Hope wheat crosses with susceptible and resistant varieties. 8 pp. Washington, 1928. [Mimeographed.]

investigators reported in this cross a single-factor difference, or a 1:3 ratio, for the immune to the segregating and resistant strains.

Quisenberry (*39*), in a study of H-44×Minhardi, found segregation for seedling reaction in the greenhouse of approximately 1 resistant, 2 segregating, and 1 susceptible in a random selection of F_3 lines inoculated with form 60. There was evidence of the influence of minor factors on the expression of rust reaction. The same lines inoculated with form 36 behaved in a similar manner, indicating that the same factors control the reaction of the seedlings in the greenhouse to these two forms of stem rust. However, little relation was found between seedling reaction in the greenhouse to these two forms and the reaction of the mature plants in the field to a collection of forms. He concluded that genetic factors, in addition to those controlling the reaction in the seedling stage, were necessary to explain the reaction in the mature-plant stage.

Goulden, Newton, and Brown (*19*) studied the reaction of seedlings and mature plants of several varieties to 16 physiologic forms of stem rust. They concluded that there were two main groups of varieties: (1) Those in which there was good agreement between seedling and mature-plant reaction, and (2) those in which there was lack of agreement between the seedling and mature-plant reaction.

Goulden (*17*) reported the transference of the resistance of Pentad (durum), *Triticum durum* Desf., to selections of *T. vulgare* in crosses between Pentad and Marquis. Two F_3 lines were obtained in which resistance was homozygous and as high as that of Hope or H-44.

Neatby and Goulden (*34*) found that in a cross of Marquis×H-44 and H-44×(Marquis×Kanred, B 2-5) field resistance was governed by a single genetic factor, but they concluded that in a Marquis×Hope cross resistance was governed by two complementary factors. In the F_2 and F_3 generations of Marquillo×H-44, it appeared that, in addition to the H-44 factor for resistance, two or more factors were contributed by Marquillo. In the cross Double Cross (Marquis-Iumillo×Marquis-Kanred)×H-44, these investigators assumed that Double Cross carried two complementary factors for resistance. Two factors were concerned in the inheritance of resistance to stem rust in the cross Webster×H-44. Webster contributed a dominant factor for moderate susceptibility, which, when homozygous, was epistatic to the H-44 factor for resistance. They found an extremely low percentage of resistant plants in the F_2 generation in crosses between Marquillo and susceptible varieties. The results indicated that three or more factors were involved in some crosses.

Neatby (*33*) studied the seedling reaction of a Marquis×H-44 cross in the greenhouse and the mature-plant reaction in the field. The mature-plant reaction to a collection of forms was entirely independent of the seedling reaction to a limited number of forms. In a Marquillo×H-44 cross the factors governing seedling reaction in the greenhouse either were expressed in the field reaction or were linked with those for mature-plant reaction. In a cross of Garnet×Double Cross the evidence obtained suggested that there was little or no relation between the factors affecting seedling reaction in the greenhouse and the mature-plant reaction in the field.

Resistance of seedlings in the greenhouse and of mature plants in the field may be due to different causes, each controlled by inde-

pendent genetic factors. Three types of resistance are known—physiological, morphological, and functional.

Stakman (*40*) has shown that resistance in the seedling stage to physiologic forms of *Puccinia graminis tritici* is due to a physiologic incompatibility between the resistant plants and the invading fungus. The nature of the incompatibility or antagonism between the cereal host and the fungus is not known.

Hursh (*29*) showed that resistance of wheat varieties to *Puccinia graminis* may be due to morphological characters. The pathogene may enter the host plant and develop normally, but the structural characters of the host may prevent extensive development of the rust. The rust mycelium develops almost exclusively in the chlorenchymatous collenchyma of the wheat stem. In morphologically resistant varieties of wheat the collenchyma bundles are small isolated strands, separated by broad bands of sclerenchymatous fibers. This forms a definite mechanical limitation to the spread of the rust mycelium. Morphologically resistant varieties therefore have a large amount of sclerenchyma and a small amount of collenchyma. The relative amount of sclerenchyma increases as the plant grows older.

Hart (*21*, *22*) confirmed and amplified Hursh's conclusions on morphological resistance, and suggested that stomatal behavior of wheat varieties during the early morning hours might be related to stem-rust resistance. She found that the stomata of certain varieties, such as Hope and Webster and certain hybrid lines derived from crosses involving these and certain other varieties, remained closed in the morning later than did those of the susceptible varieties and hybrid lines. This type of resistance was called "functional resistance", and Hart suggests that in some cases mature-plant resistance may be partly functional in nature.

Peterson (*36*) made similar observations on a number of standard varieties and of certain hybrids from two crosses, namely, H–44 × Reward and H-44 × Renfrew, and was unable to verify Hart's observations regarding the correlation between stomatal behavior and resistance to rust.

From the standpoint of practical breeding, important forward steps were taken in the production of Marquillo and Hope, two of the varieties considered in this paper. Marquillo was produced by Hayes and his associates (*23*, *25*, *27*) from a cross of Marquis and Iumillo durum, and Hope was produced by McFadden (*31*) from a cross of Marquis and Yaroslav emmer (*Triticum dicoccum* Schrk.) Marquillo has proved moderately to highly resistant under field conditions and Hope is highly resistant in the mature-plant stage to many and perhaps all rust forms of the Mississippi Valley, although susceptible in the seedling stage to some forms.

REACTION TO BUNT

Farrer (*11*, *12*, *13*), of Australia, began in 1901 to develop bunt-resistant varieties of wheat by hybridization, but made no attempt to study the ratios of resistant and susceptible groups. The percentage of bunt in 10 Australian wheats ranged from 12 to 95.5, and Farrer assumed that similar degrees of infection might occur in hybrid generations of crosses of these varieties. He subjected the F_2 and F_3 generations to a heavy bunt attack and selected the bunt-

free plants. Hybrid selections were obtained that had 5 to 10 percent of bunt, whereas the susceptible varieties had 80 to 100 percent. In 1905 Farrer made crosses for the specific purpose of getting bunt-resistant varieties. After Farrer's death his assistant, G. L. Sutton, carried on the work and produced two highly resistant varieties, Florence and Genoa.

Pye (*38*) made numerous crosses between Medeah, a highly resistant durum, and *Triticum vulgare* wheats. It was not difficult to obtain resistant hybrids but none of these met the practical requirements of the farmer and miller.

Gaines (*15*) studied the genetics of resistance in the following types of crosses: Resistant × resistant, resistant × susceptible, and susceptible × susceptible. The most susceptible wheats, sown under conditions favoring maximum infection, had an average of 80 percent of bunted heads. Gaines concluded that the freedom from bunt of the remaining 20 percent was due to escapes, for the descendants of these in-crosses showed no evidence of having inherited resistance. Crosses between susceptible varieties produced only susceptible offspring, whereas crosses between resistant varieties produced progeny showing transgressive segregation. Lines were obtained from such crosses that segregated for immunity, resistance, and susceptibility. Gaines studied more than 25 separate crosses and explained the results obtained in each on a multiple-factor basis.

Gaines and Singleton (*16*) assumed that in a cross of Turkey × Marquis two factors controlled resistance, the one carried by Turkey being much more potent than the one carried by Marquis.

Briggs (*5*) made three types of crosses, namely, susceptible × susceptible, resistant × resistant, and resistant × susceptible. The resistant parents were Martin and Hussar, which produced no bunt. The susceptible ones were Hard Federation, Baart, and White Federation, which produced 50 to 95 percent of bunt. He concluded that Martin differed from such susceptible varieties as Hard Federation and White Federation in one dominant factor for resistance. Later (*7*) he reported that Hussar differed from Hard Federation and Baart by two dominant factors. One of these factors was identical with the factor in Martin but the other was unlike the completely dominant Martin factor, as it permitted about 50 percent of the heterozygous plants to become infected when the Martin dominant factor was absent. Briggs (*6*) later reported that White Odessa had a single dominant factor for resistance similar in its effect to the Martin factor. It was not known whether this factor was identical with the Martin factor. He found (*8*) that Banner Berkeley differed from White Federation in one main factor for resistance to bunt. This factor was identical with the one present in Martin.

Aamodt (*3*) studied the inheritance of resistance to bunt in the F_3 of the following types of crosses of spring-wheat varieties: (1) Susceptible × susceptible, (2) moderately susceptible × moderately susceptible, (3) susceptible × moderately resistant, (4) moderately susceptible × moderately resistant, and (5) moderately resistant × moderately resistant. All degrees of infection were obtained, and in every cross studied a number of F_3 lines transgressed beyond the range shown by either parent. This showed that several genetic factors controlled the reaction to bunt and that numerous recombinations had been produced. Aamodt concluded that multiple

factors, the exact nature of which had not been determined, governed the reaction to bunt.

PARENTAL MATERIAL

The three common wheat varieties, Hope, Marquillo, and Supreme, were used as parents in this study. Parental material was obtained from single-head selections from the rod-row trials of each of the varieties grown at the United States Northern Great Plains Field Station, Mandan, N.Dak., in 1927. Table 1 shows the comparable characters of the three varieties.

TABLE 1.—*Comparable characters of 3 wheat varieties*

Character	Hope	Marquillo	Supreme
Reaction to—			
Stem rust	Highly resistant	Moderately resistant	Susceptible.
Bunt	Resistant	Semiresistant	Do.
Black chaff	Susceptible	Resistant	Resistant.
Awnedness	Awned	Awnleted	Awnless.
Coleoptile color	Purple	Green	Green.

METHODS

Reciprocal crosses were made in 1928. One half of the F_1 plants of each of the three crosses were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D.C.), in the winter of 1928–29. The other half of the F_1 plants and the F_2 plants from the greenhouse were grown at the Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minn., in 1929. In order to obtain as much seed as possible from each plant, the kernels were spaced 6 inches apart in rows 1 foot apart. An F_3 line was grown from each F_2 plant.

The F_3 generation was grown at University Farm, St. Paul, Minn., in 1930. From each F_2 plant duplicate 6-foot rows, containing 25 seeds each, were space-planted for the rust study. An F_2 population was also grown in 1930 from seed obtained from the F_1 plants grown in 1929. Checks of the parents of each cross were grown in every twentieth row.

STEM RUST

Artificial epidemics of stem rust were produced at University Farm, in 1930 and 1931, by increasing physiologic forms of the rust in the greenhouse and hypodermically inoculating at heading time plants of susceptible wheat varieties in the border and alley rows in the field with a water suspension from a mixture of urediospores of all available forms of the rust. Seventeen physiologic forms were used. Separate inoculations with each individual form were also made in the field, but the relative number or prevalence of the different forms on the mature plants at harvest was not determined. Additional infection was induced by similarly inoculating susceptible plants in the heading stage in the greenhouse with all available physiologic forms of rust and transferring them to the field, where they were spaced alternately with inoculated plants at intervals of approximately 20 feet in the alleys and border rows.

Tests of seedling reaction to stem rust, physiologic form 36, were made in the greenhouse. The method of making these tests was similar to that described by Aamodt (*1*) and by Hayes, Stakman, and Aamodt (*27*). The seedlings were inoculated when the first leaf was about 2 inches long.

In these tests duplicate seedings of 20 plants to each 4-inch pot were made. Tests were made of all F_3 lines after taking out the seed necessary for the field tests.

In the study of stem-rust reaction in the field the F_2 plants and the plants of each F_3 line were placed in one of the three classes *R*, *SR*, and *S*. *R* (resistant) includes plants with no infection or only a trace of rust, having a small or narrow linear type of pustule; *SR* (semiresistant) includes plants that appear less resistant than those in *R* and have a type of pustule smaller than that of *S*, the susceptible type; and *S* (susceptible) includes plants having an abundance of rust and well-developed, large pustules that coalesce. The F_3 lines were then grouped according to breeding behavior into the following five classes:

R = Breeding true for *R*
R— = Segregating for *R* and *SR* or breeding true for *SR* type
H = Segregating for *R*, *SR*, and *S*
S— = Segregating for *SR* and *S*
S = Breeding true for *S*

BUNT

In a study of bunt reaction a separate planting was made of F_3 lines infected with the collection of bunt obtained from R. H. Bamberg. No attempt was made to determine the number of physiologic forms present. Twenty-five kernels of wheat were placed in an envelop. After adding a small spoonful of spores, the envelop was shaken until the kernels were completely blackened.

The number of bunted heads was divided by the total number of heads in each line, the results showing the percentage of infection.

BLACK CHAFF

For a black-chaff study an attempt was made to induce an artificial epidemic in the cereal pathology garden, but a better epidemic developed naturally on the material grown in the rust nursery; hence the data on black chaff were obtained from the latter material.

Black-chaff infection was recorded as heavy, medium, and light. The F_3 lines were then grouped according to breeding behavior into the following five classes:

H = Breeding true for heavy infection
H— = Containing plants with heavy and medium infection
S = Segregating for heavy, medium, and light infection
L— = Containing plants with medium and light infection
L = Breeding true for light infection

EXPERIMENTAL RESULTS

REACTION TO STEM RUST

Studies were made in the field on F_2, F_3, and F_4 material and in the greenhouse on F_3 lines. The field studies in 1930 included F_3 families from the entire F_2 population grown the previous year. Some F_4 selections were grown in 1931 to test the accuracy of the F_3 classification.

Field Studies

Field notes were taken on mature plants, and are referred to herein as mature-plant reaction. The plants in each row were pulled, tied, wrapped, and carefully labeled. The bundles were then shocked under canvas covers to protect the plants from the weather, and the notes were taken the following month.

Table 2.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of the Hope×Marquillo wheat cross, 1930 and 1931*

Parent or generation	Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
			R	*R*−	*SR*	*H*	*S*−	*S*	Total
Hope.....plants	1930	.....	69	.....	.....	.....	.....	.....	69
Marquillo.....do	1930	.....	10	.....	38	.....	.....	.....	48
F_2.....do	1930	.....	110	.....	214	.....	.....	112	436
Hope.....rows	1930	.....	11	.....	.....	.....	.....	.....	11
Marquillo.....do	1930	.....	.....	11	.....	.....	.....	.....	11
F_3.....lines	1930	.....	33	73	.....	67	22	7	202
Hope.....rows	1931	*R*	5	.....	.....	.....	.....	.....	5
Marquillo.....do	1931	*R*	.....	.....	.....	.....	1	.....	1
Do.....do	1931	*SR*	.....	.....	.....	.....	4	.....	4
F_4.....lines	1931	*R*	13	9	.....	13	8	.....	43
Do.....do	1931	*SR*	.....	4	.....	9	4	18	35
Do.....do	1931	*S*	.....	.....	.....	3	3	24	30

In table 2 are presented data on mature-plant reaction to stem rust in the parent and F_2 plants and in the parent rows and F_3 and F_4 lines of the Hope×Marquillo cross. In considering table 2 it should be noted that the data in the upper three lines pertain to single plants while those below pertain to rows As indicated on page 37, the designation of the plants is somewhat different from that of the rows.

In 1930, all the plants of the Hope parent, grown adjacent to the F_2 plants, were classed as resistant, whereas the plants of Marquillo, similarly grown, were recorded as 10 resistant and 38 semiresistant. There were 436 F_2 plants of the Hope×Marquillo cross, 110 of which were classed as resistant, 214 as semiresistant, and 112 as susceptible, the latter class being unlike the parents (fig. 1). Although in this experiment Hope was more highly resistant than Marquillo, it was not possible to differentiate clearly between the two types of resistance in the F_2 generation. As Marquillo produced both *R* and *SR* plants in the proportion of about 1 to 4, it is probable that 1 out of each 5 plants in the F_2 resistant group may show the Marquillo type of resistance.

All the rows of the Hope parent, grown adjacent to the F_3 lines of the Hope×Marquillo cross, were classified as resistant, whereas all the rows of the Marquillo parent, similarly grown, were placed in the *R*− group. Nine of the Marquillo check rows had a ratio of 1 resistant to 4 semiresistant plants, and two rows had all plants classified as semiresistant.

The 202 F_3 lines were grouped as follows: 33, *R*; 73, *R*−; 67, *H*; 22, *S*−; and 7, *S*. According to this classification, 33 lines were as resistant as the Hope parent and 106 were within the limits of both parents, whereas 96 exceeded the reaction of both parents.

The results of the studies on the reaction to stem rust in both the F_2 and F_3 generations show that the factor or factors for the mature-plant resistance of the Hope type are not allelomorphic to those of the semiresistant type of Marquillo, as types were obtained that were more susceptible than the semiresistant parent.

A further test of the mode of inheritance of the Hope type of resistance in this cross was made by comparing the number of resistant

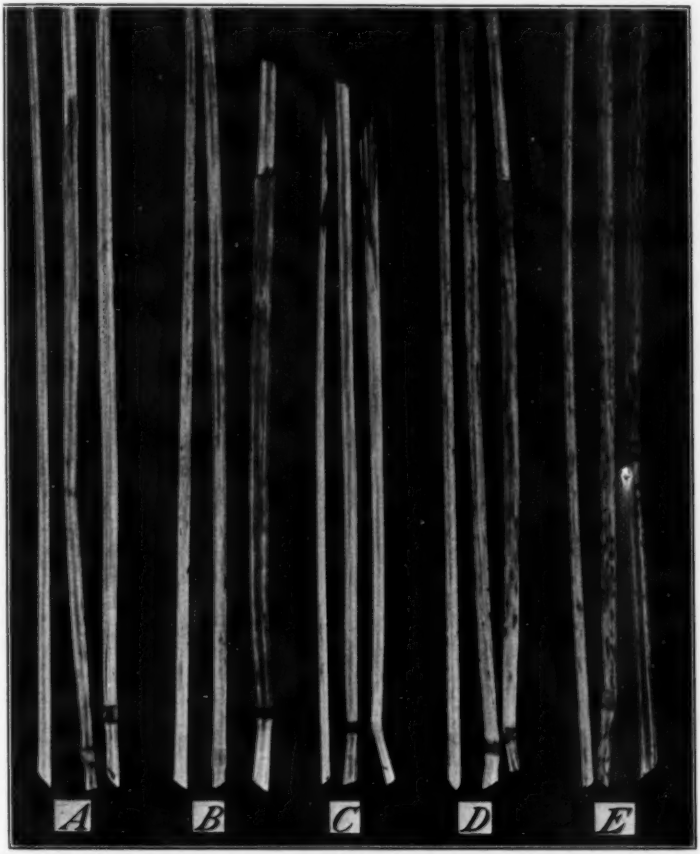


FIGURE 1.—Mature plant reaction to a collection of physiologic forms of stem rust in the field in cross of Hope×Marquillo wheats. Parents: *A*, Hope, resistant; *B*, Marquillo, semiresistant. Segregation of F_2 plants and plants of F_3 lines of Hope×Marquillo cross: *C*, Resistant; *D*, semiresistant; *E*, susceptible.

plants with the combined number of semiresistant and susceptible plants of the individual F_3 *H* segregating lines, according to the method described by Kirk and Immer (*30*). The χ^2 was calculated for each individual F_3 line for a given ratio of resistant to semiresistant and susceptible plants. If the value of χ^2 for a line gave a *P* value of more than 0.05 (*14, p. 96*), the F_3 line was placed in that particular group.

In each of the individual segregating F_3 lines, the data for the observed number of plants in the resistant group as compared with the observed number in the combined semiresistant and susceptible groups were compared with numbers calculated for the ratios 1:3, 1:15, and 9:7.

According to this method of analysis of the 67 F_3 lines segregating for resistant to semiresistant and susceptible plants 50 segregated in a 1:3 ratio, 8 in a 1:15 ratio, and 9 in a 9:7 ratio.

The F_3 lines segregating in a 1:3 ratio would result from a single-factor difference for resistance, and those segregating in a 1:15 ratio would result from duplicate factors for susceptibility. A 9:7 ratio could be obtained from the interaction of complementary factors for resistance. These types of segregation indicate that three or more factors are concerned in the inheritance of rust reaction in this cross. There were too many lines segregating in a 9:7 ratio to be explained by natural crossing. This abnormal ratio may be due, however, to the abnormal chromosomal behavior of Marquillo as found by Powers (*37*), who concluded that Marquillo possesses greater germinal instability than Marquis. Hope is a derivative of a species cross (*31*) and may also exhibit variability in chromosomal behavior.

Other investigators have pointed out the complexity of the problem of studying the reaction to rust as well as to other diseases, as both the host and the pathogene must be considered and both are influenced by environmental conditions.

In 1931 the reaction to stem rust in certain F_3 plants from segregating F_3 rows was studied in their F_4 progeny. All the rows of the Hope parent, grown adjacent to the F_4 progenies, were classed as resistant. One plant of the Marquillo parent classified as resistant and four plants classified as semiresistant in 1930 produced both semiresistant and susceptible plants in 1931. This greater susceptibility of the Marquillo parent in 1931 may have been due to the presence of different physiologic forms in the field or to the influence of environment on the factors governing resistance. The progeny of the F_3 *R* plants of the Hope×Marquillo cross contained approximately 1 homozygous line to 3 segregating for 2 or for all 3 of the rust classes. Of the semiresistant plants 4 lines bred true for the *R*− group, but a majority segregated for all classes or bred true for susceptibility. This segregation shows the complexity of the Marquillo type of resistance. The *S* type of plants for the most part bred true.

TABLE 3.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of Hope×Supreme wheat cross, 1930 and 1931*

Parent or generation	Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
			R	*R*−	*SR*	*H*	*S*−	*S*	Total
Hope.....plants..	1930		37						37
Supreme.....do....								53	53
F_2.....do....			228		610			360	1,198
Hope.....rows..			6						6
Supreme.....do....								6	6
F_3.....lines..			6	29		36	25	7	103
Hope.....rows..	1931	*R*	8						8
Supreme.....do....		*S*						8	8
F_4.....lines..		*R*	10	22		7	8		47
Do.....do....		*SR*	1	12		2	20	16	51
Do.....do....		*S*		1			1	29	31

The data in table 3 show the mature-plant reaction to stem rust of F_2 plants and F_3 and F_4 lines of the Hope × Supreme cross, together with the reaction to stem rust of the two parents. These data are graphically presented in figure 2.

When the results obtained in the F_3 generation are compared by the χ^2 method with the calculated number based on a 2-factor difference, $\chi^2 = 11.7524$ and $P = 0.04$. This means that on the basis of random sampling a deviation as great as or greater than that observed could be expected only 4 times out of 100 trials. The agreement between the calculated and the observed ratios is therefore poor in

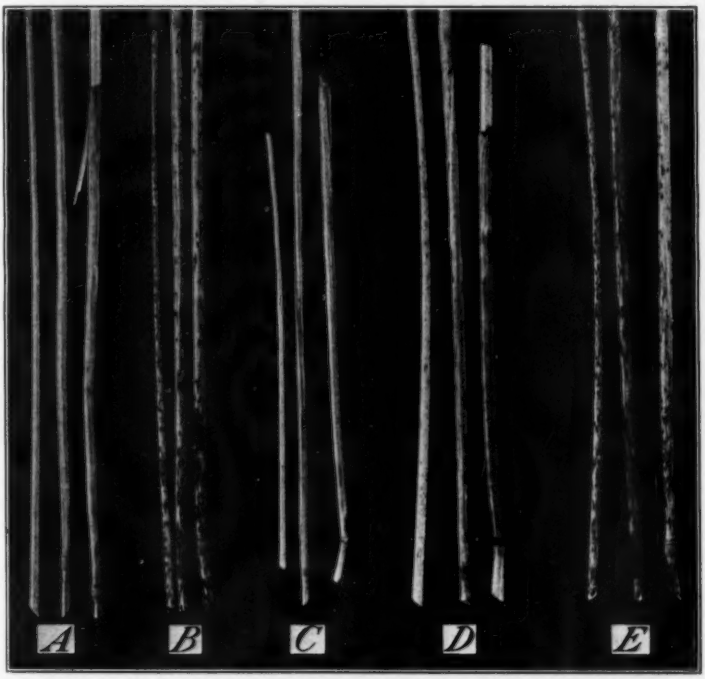


FIGURE 2.—Mature-plant reaction to a collection of physiologic forms of stem rust in the field in cross of Hope×Supreme wheats. Parents: *A*, Hope, resistant; *B*, Supreme, susceptible. Segregation of F_2 plants and plants of F_3 lines of Hope×Supreme cross: *C*, Resistant; *D*, semiresistant; *E*, susceptible.

that there were too few lines breeding true for semiresistance and too many lines segregating for all three classes. It is also possible that difficulties in classifying these two groups may partly account for the poor fit obtained. Despite the small number of F_3 lines tested, the results, as a whole, indicate the presence of at least two major genetic factors for rust reaction.

An analysis of the segregating F_3 *H* lines was made by applying the goodness-of-fit test, as in the cross previously discussed.

Of the 36 F_3 lines segregating for all three classes, 21 lines appeared to segregate for resistant and combined semiresistant and susceptible plants in a ratio of 1:3, and 13 lines in a ratio of 1:15. The *P* values

obtained for both types of segregation show a very good fit to a calculated 1:3 and 1:15 ratio, respectively. Two lines that approached a 9:7 ratio, according to this test, may have resulted from natural crossing or mechanical mixtures. The number of lines segregating in the two ratios 1:3 and 1:15 supports the conclusion that at least two genetic factors are involved in the inheritance of stem-rust resistance in this cross.

In 1931 the progeny from *R* F_3 plants from segregating lines included 10 resistant lines and 37 lines segregating for 2 or 3 classes. The progeny of *SR* F_3 plants included 1 resistant line and 50 lines segregating for 2 or 3 classes or susceptible. Plants classified as *S* in the F_3 gave 1 semiresistant line, 1 line segregating for semiresistance and susceptibility, and 29 lines breeding true for susceptibility.

TABLE 4.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of the Marquillo×Supreme wheat cross, 1930 and 1931*

Parent or generation	Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
			R	*R*−	*SR*	*H*	*S*−	*S*	Total
Marquillo plants	1930		7		49				56
Supreme do								55	55
F_2 do					110			415	525
Marquillo rows				7					7
Supreme do								7	7
F_3 lines				1			60	77	138
Marquillo rows	1931	*R*		1					1
Do do		*SR*		1					1
Supreme do		*S*						2	2
F_4 lines		*SR*		2			15	2	19
Do do		*S*					2	10	12

The data in table 4 show the mature-plant reaction to stem rust of the F_2 plants and F_3 and F_4 lines of the Marquillo×Supreme cross, together with the reaction to stem rust of the two parents. These data are graphically presented in figure 3.

No plants as resistant as the more resistant plants of the Marquillo parent were obtained in the F_2 generation.

All seven rows of the Marquillo parent were classified as *R*−, each having a few resistant plants. The breeding behavior of the 138 F_3 lines is clearly shown in the table. Although the resistance of the individual plants classified as *SR* was fairly high, it was not equal to that of Marquillo.

Hayes, Stakman, and Aamodt (*27*) concluded from a study of F_3 lines of a Marquillo×(Marquis×Kanred) cross that the resistance of Marquillo was governed by two main factors but that modifying factors also may operate. The results obtained in the experiments herein reported indicate the presence of at least three factors. Seventeen physiologic forms of stem rust were used in the artificial epidemic induced by the writer, while only nine were used by Hayes and Aamodt (*24*). The lower proportion of resistant plants may have been due to the presence of the larger number of physiologic forms or to the nature of the cross. These results and conclusions corroborate those obtained by Neatby and Goulden (*34*) with crosses between Marquillo and susceptible varieties of wheat.

In 1931, two rows each of the Marquillo and Supreme parents were grown. One plant of Marquillo previously classified as *R* and another as *SR* produced similar reactions, both being classified as *R*−,

FIGURE 3.—Mature-plant reaction to a collection of physiologic forms of stem rust in the field in cross of Marquillo×Supreme wheats. Parents: *A*, Marquillo, semiresistant; *B*, Supreme, susceptible. Segregation of F_2 plants and plants of F_3 lines of Marquillo×Supreme cross: *C*, Semiresistant; *D*, susceptible.

while the two rows of Supreme classed as *S* in 1930 produced only susceptible plants in 1931. The progeny of the F_3 *SR* plants included 2 lines classified as *R*−, 15 as *S*−, and 2 as *S*. The *S* F_3 plants gave 2 lines classified as *S*− and 10 lines that bred true for *S*.

GREENHOUSE STUDIES

The seedling reaction to form 36 in the three parent varieties and in the F_3 hybrids of the Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme crosses was studied in the greenhouse. Hope is highly resistant to form 36 in the seedling stage, and all 296 plants

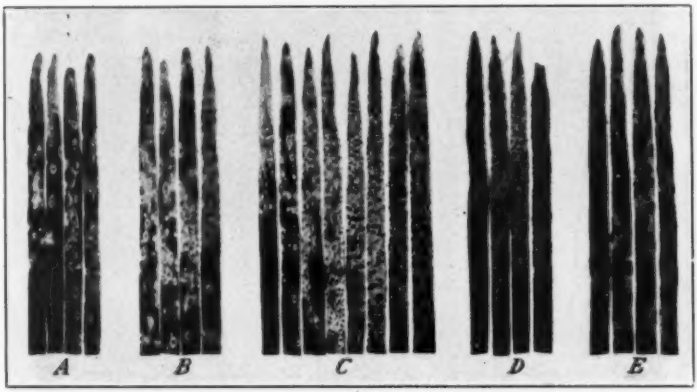


FIGURE 4.—Seedling reaction in the greenhouse to stem rust, form 36, in cross of Hope×Marquillo wheats: *A*, Hope parent, resistant. F_3 of Hope×Marquillo cross: *B*, Resistant; *C*, segregating; *D*, susceptible. *E*, Marquillo parent, susceptible.

were classed as resistant. All the Marquillo plants were infected, a majority of the pustules being of type 4, indicating susceptibility. Supreme also was susceptible, all plants having the type 4 pustule. All the F_3 lines of the Marquillo×Supreme cross were susceptible.

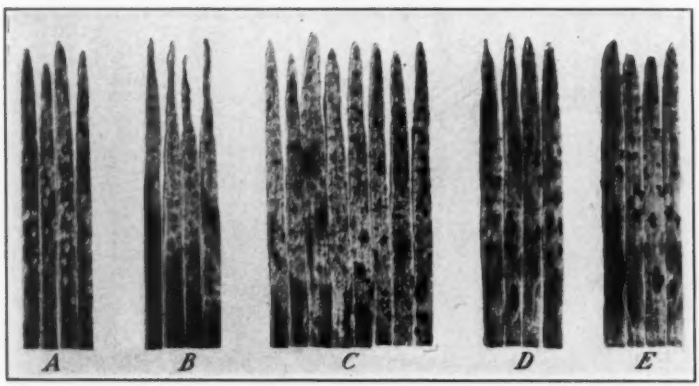


FIGURE 5.—Seedling reaction in the greenhouse to stem rust, form 36, in cross of Hope×Supreme wheats: *A*, Hope parent, resistant. F_3 of Hope×Supreme cross: *B*, Resistant; *C*, segregating; *D*, susceptible; *E*, Supreme parent, susceptible.

The seedling reaction in the two crosses Hope×Marquillo and Hope×Supreme was fairly distinct, so that it was comparatively easy to separate the F_3 lines into three groups—resistant, semiresistant, and susceptible (figs. 4 and 5). Table 5 shows the seedling reaction to form

36 in parent rows and F_3 lines of the three crosses Hope × Marquillo, Hope × Supreme, and Marquillo × Supreme.

TABLE 5.—*Seedling reaction to stem rust, form 36, in parent plants and in F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats, grown in the greenhouse in 1930*

Parent or cross	Number of plants or lines showing indicated reaction			
	Resistant	Segregating	Susceptible	Total
Hope ..... plants	296			296
Marquillo ..... do			130	130
Supreme ..... do			137	137
Hope × Marquillo ..... lines	46	88	31	165
Hope × Supreme ..... do	29	46	16	91
Marquillo × Supreme ..... do			130	130

In the Hope × Marquillo cross the numbers, compared with a calculated 1:2:1 ratio, gave $P = 0.18$. In the Hope × Supreme cross the numbers, compared with a calculated 1:2:1 ratio, gave $P = 0.16$. These P values and the fact that only one type of segregation was observed in the heterozygous lines indicate that a single genetic factor difference is responsible for the results.

REACTION TO BUNT

Reaction to bunt was studied in the three parent varieties and in the F_3 hybrid lines of the crosses. The F_2 material was not bunted. The parents used in a particular cross were grown, alternately, every twentieth row. The results are presented in table 6.

TABLE 6.—*Distribution of bunted heads in parent rows and in F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats in 1930*

Parent or cross	Number of rows or lines showing indicated percentage of bunt infection							
	0	0.1–3	3.1–6	6.1–9	9.1–12	12.1–15	15.1–18	Total
Hope ..... rows	16	1						17
Marquillo ..... do	7	8	3					18
Supreme ..... do	1	2	3	3	2		2	13
Hope × Marquillo ..... lines	117	66	12	3				198
Hope × Supreme ..... do	37	45	12	7	1	1		103
Marquillo × Supreme ..... do	12	61	34	16	5	3		131

Hope was highly resistant, only 1 row of 17 showing any infection. In the case of the Marquillo parent there was slightly more infection. Supreme was the most susceptible parent, having a greater amount of bunt and a greater range of variability than either Hope or Marquillo. One row of Supreme was bunt free; in others, there was infection in varying amounts up to 18 percent.

The bunt data on the F_3 hybrid lines show segregation approximately covering the range of the respective parents. In the Hope × Marquillo cross 183, or 92.4 percent, of the F_3 lines were within the limits of the Hope parent, and all but 3 were within the limits of both parents. Of the 103 F_3 lines of the Hope × Supreme cross 82, or 79.6 percent, were

within the limits of the Hope parent. The bunt reaction in these two crosses was very similar except that there was greater susceptibility in the cross with Supreme, the more susceptible parent. From these crosses with Hope as one of the parents, 154 of 301 F_3 lines grown were bunt free.

Of 131 F_3 lines of the Marquillo × Supreme cross, 12 were bunt free and 107 were within the limits of Marquillo, the more resistant parent. More lines with higher percentages of bunt were obtained in this cross, as might be expected, since neither parent is as resistant as the Hope variety used in the two other crosses.

The results from the three crosses indicate that the greater the susceptibility of one or both of the parents used in a particular cross the greater is the tendency toward susceptibility in the hybrids. Because of the small amount of bunt obtained it is impossible to determine the number of genetic factors involved in the reaction to bunt in the crosses.

REACTION TO BLACK CHAFF

Black chaff affects various parts of the spike of the wheat plant and any or all parts of the culm. The disease sometimes may cause serious losses in the wheat crop. Waldron (*43*) found a decrease in weight of grain and in fertile culms per plant as infection by black chaff increased. In crosses of Hope wheat with other varieties he found an "antagonistic relationship" between resistance to stem rust and resistance to black chaff.

A study of black-chaff reaction is very difficult because little is known of the conditions favoring the development of an epidemic. There usually is a wide range of variability in the reaction of wheat varieties in different parts of a nursery and in different seasons. The data obtained on black-chaff infection in 1930 are presented in table 7.

TABLE 7.—*Reaction to black chaff in parent and F_2 plants and in parent rows and F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats in 1930*

Parent or cross and generation	Number of plants, rows, or lines showing indicated reaction						
	H	*H*–	*M*	*S*	*L*–	*L*	Total
Hope.....plants	2	.....	18	.....	.....	96	116
Marquillo.....do	.....	.....	.....	.....	.....	101	101
Supreme.....do	.....	.....	.....	.....	.....	86	86
Hope×Marquillo F_2.....lines	15	.....	83	.....	.....	338	436
Hope×Supreme F_2.....do	7	.....	99	.....	.....	1,092	1,198
Marquillo×Supreme F_2.....do	.....	.....	.....	.....	.....	525	525
Hope.....rows	.....	.....	.....	2	8	7	17
Marquillo.....do	.....	.....	.....	.....	1	17	18
Supreme.....do	.....	.....	.....	.....	.....	13	13
Hope×Marquillo F_3.....lines	.....	.....	.....	11	83	104	198
Hope×Supreme F_3.....do	.....	.....	.....	3	36	64	103
Marquillo×Supreme F_3.....do	.....	.....	.....	.....	.....	138	138

The infection of Hope, the susceptible parent, grown adjacent to the F_2 plants and the F_3 lines shows the variability of the epidemic in different parts of the nursery. Segregation appeared to occur in the F_2 generation of the two crosses having Hope as one of the parents, although the infection in most of the plants was classed as light. Definite segregation was less apparent in the F_3 generation, the infection in the larger percentage of the lines being classed as light, as in the Marquillo and Supreme parents.

All plants in the F_2 and F_3 generations of the Marquillo × Supreme cross had a light infection of black chaff and showed no segregation.

AWNEDNESS

The awn development of the F_2 plants and the F_3 lines in the triangular crosses of Hope, Marquillo, and Supreme was studied in 1930. As stated, all the F_2 plants were tested in the F_3 generation in all of the crosses insofar as there was available seed.

HOPE × MARQUILLO

The F_1 hybrids of the Hope × Marquillo cross had awnlets slightly longer than those of the Marquillo parent. The F_2 and F_3 plants grown in 1930 were described in three classes, namely, awned like Hope, strongly awnleted like the F_1, and awnleted like Marquillo. The numbers obtained in the F_2 and F_3 generations were compared with the calculated numbers in a 1 : 2 : 1 ratio by the χ^2 or goodness-of-fit method, giving P values of 0.86 and 0.33, respectively. The results obtained in both the F_2 and F_3 generations, therefore, show a single genetic factor difference for awnedness between the two varieties, Hope and Marquillo. This single-factor difference is similar to that shown by Biffen in 1905 (*4*) and by many recent investigators.

HOPE × SUPREME

In the Hope × Supreme cross and its reciprocal the F_1 plants were apically awnleted or more nearly like the awnless parent. The F_2 plants were grouped in three classes: (1) Awnless like Supreme; (2) a heterozygous group with awns varying in length between the two parent varieties; and (3) awned like the Hope parent. Awn types of the F_3 lines were placed in six groups: (1) Awnless; (2) segregating awnless to awnleted; (3) awnleted; (4) segregating for all types from awnless to awned; (5) segregating awnleted to awned; and (6) awned. The results obtained in the F_2 and F_3 generations showed a very satisfactory fit to a two-factor difference for awnedness when similar numbers were compared by the χ^2 or goodness-of-fit method, P values of 0.12 and 0.07 being obtained.

The assumption of a two major factor difference between crosses of an awned and an awnless wheat is in accord with that of Howard and Howard (*28*), Clark (*9*), Clark, Florell, and Hooker (*10*), and Stewart and Heywood (*41*).

MARQUILLO × SUPREME

The F_1 plants of this cross were apically awnleted and more nearly like the awnless parent. The F_2 plants were placed in two classes: (1) Awnleted like the Marquillo parent, and (2) all plants having shorter awns to awnless, or similar to those of the Supreme parent. It was difficult to separate the awnless and apically awnleted plants because of the variation in a single plant. Therefore, in analyzing the data, these two classes were grouped together. The results in both the F_2 and F_3 generations show a single major factor difference for awnedness between Marquillo and Supreme, when the numbers are compared by the goodness-of-fit method. A P value of 0.40 was obtained in both cases. These results are in accord with those obtained by Stewart and Tingey (*42*).

COLOR OF COLEOPTILE

The first foliage leaf to appear when the young plant emerges fro the soil is enclosed in the plumule sheath, or coleoptile. According to Percival (*35*) the coleoptile of wheat may be pale green, colorless, or pink.

The coleoptiles of Hope wheat plants are purple. This color persists, as in H-44 as described by Quisenberry (*39*), after the foliage leaves grow out of the coleoptile; and in some cases the purple tinge shows at the base of the first leaf. Marquillo and Supreme have distinctly pale-green coleoptiles. Data were obtained on the color of the coleoptile of the parents and hybrids in the two crosses of which Hope is one of the parents. Approximately 40 plants were available for the study of each of the hybrid lines, and, in addition, a large number of parent plants were classified. The intensity of coleoptile color was variable and appeared to be influenced by temperature and sunlight at the time of germination and emergence. All the data for an F_3 line were combined to determine the breeding behavior of this character. The plants were placed in two classes only—purplish or green. All Hope plants were classed as purple and those of Marquillo and Supreme as green. A comparison of the numbers obtained for purple, segregating, and green, with the calculated number based on the 1 : 2 : 1 ratio, gives a P value of 0.48. This is a satisfactory fit. Both crosses with Hope as one parent indicate that a single factor is involved in the inheritance of color of coleoptile. In the Marquillo × Supreme cross all plants of the F_3 lines were green.

INDEPENDENCE OF REACTION IN THE CHARACTERS STUDIED

A study was made of the interrelations of the various characters. A measure of the relationship between the distribution of two characters was obtained by calculating χ^2 for independence and by determining the value of P from Fisher's table (*14*). To find the value of P from this table, Fisher's plan in regard to the number of degrees of freedom has been followed. In a fourfold by threefold table in which r = rows and c = columns, $n = (r-1)(c-1)$, or six degrees of freedom. The P value is obtained for six degrees of freedom and for the calculated value of χ^2.

The studies of the interrelations of reaction to stem rust (both seedling and mature-plant reaction), bunt, black chaff, awnedness, and coleoptile color are summarized in table 8.

All characters were inherited independently, with the exception of stem rust (field) and black chaff, and stem rust (field) and seedling reaction in the Hope × Marquillo cross. In the reaction of this cross to stem rust there was a tendency to association, probably genetic, of the mature-plant reaction in the field to a collection of physiologic forms with the seedling reaction in the greenhouse to form 36. The data showing this relationship are presented in table 9.

TABLE 8.—*Independent inheritance of the characters in the F_2 and F_3 wheat crosses of Hope, Marquillo, and Supreme*

Cross and generation	Number of plants or lines	Characters compared	n	χ^2	P
Hope×Marquillo F_2	plants 436	Stem rust (field) and black chaff	4	164.09	(a)
Hope×Marquillo F_3	lines 202	do	8	16.64	0.04
Hope×Supreme F_2	plants 1,198	do	4	151.57	(a)
Hope×Supreme F_3	lines 103	do	4	11.25	.03
Hope×Marquillo F_2	plants 436	Stem rust (field) and awnedness	4	2.57	.43
Hope×Marquillo F_3	lines 202	do	8	3.50	.90
Hope×Supreme F_2	plants 1,198	do	4	1.10	.90
Hope×Supreme F_3	lines 103	do	20	12.40	.90
Marquillo×Supreme F_2	plants 525	do	1	.65	.44
Marquillo×Supreme F_3	lines 138	do	3	1.51	.68
Hope×Marquillo F_2	do 198	Stem rust (field) and bunt	12	12.61	.40
Hope×Supreme F_2	do 103	do	9	9.85	.37
Marquillo×Supreme F_2	do 131	do	4	1.58	.81
Hope×Marquillo F_2	do 165	Stem rust (field) and seedling reaction	6	17.22	(a)
Hope×Supreme F_2	do 91	do	8	6.64	.58
Hope×Marquillo F_2	do 165	Stem rust (field) and color of coleoptile	6	6.33	.40
Hope×Supreme F_2	do 91	do	8	6.69	.88
Hope×Marquillo F_2	do 198	Bunt and awnedness	6	2.24	.86
Hope×Supreme F_2	do 103	do	10	14.39	.16
Marquillo×Supreme F_2	do 131	do	8	1.95	.98
Hope×Marquillo F_2	do 164	Bunt and seedling reaction	4	3.79	.44
Hope×Supreme F_2	do 91	do	6	6.01	.43
Hope×Marquillo F_2	do 164	Bunt and color of coleoptile	6	1.53	.96
Hope×Supreme F_2	do 91	do	6	1.82	.93
Hope×Marquillo F_2	plants 436	Black chaff and awnedness	4	2.98	.57
Hope×Marquillo F_3	lines 202	do	4	6.85	.15
Hope×Supreme F_2	plants 1,198	do	4	2.32	.68
Hope×Supreme F_3	lines 103	do	5	7.14	.22
Hope×Marquillo F_2	do 198	Black chaff and bunt	2	.42	.81
Hope×Supreme F_2	do 103	do	3	2.43	.49
Hope×Marquillo F_2	do 165	Black chaff and seedling reaction	4	3.24	.52
Hope×Supreme F_3	do 100	do	2	.33	.62
Hope×Marquillo F_2	do 165	Black chaff and color coleoptile	4	6.49	.16
Hope×Supreme F_2	do 91	do	2	3.87	.16
Hope×Marquillo F_2	do 165	Seedling reaction and awnedness	4	8.08	.09
Hope×Supreme F_3	do 90	do	10	16.11	.13
Hope×Marquillo F_2	do 165	Color coleoptile and seedling reaction	4	4.51	.35
Hope×Supreme F_2	do 91	do	4	4.36	.37
Hope×Marquillo F_2	do 165	Color of coleoptile and awnedness	4	3.27	.52
Hope×Supreme F_2	do 91	do	10	7.48	.68

a Less than 0.01.

TABLE 9.—*Relation between seedling reaction to stem rust, form 36, in the greenhouse and mature-plant reaction to 17 forms in the field in F_3 lines of the Hope×Marquillo wheat cross*

Seedling reaction to form 36 in greenhouse	Number of lines showing indicated mature-plant reaction to 17 forms of stem rust in field				
	R	*R*−	*H*	*S*− and *S*	Total
R	13	14	13	6	46
SR	7	39	33	9	88
S	4	6	14	7	31
Total	24	59	60	22	165

P=less than 0.01.

Of the 24 lines resistant in the field, 13 were resistant to form 36 in the greenhouse, and of the 22 lines susceptible in the field, 6 were resistant to form 36 in the greenhouse. The value of *P* is less than 0.01, showing that the two reactions are not independent. These results are similar to those obtained by Neatby (*33*).

In the two crosses Hope×Marquillo and Hope×Supreme, there appeared to be a tendency to linkage between reaction in the field and to stem rust and to black chaff. This tendency has been observed at University Farm for several years. Table 10 shows the relationship between the two diseases in the field in the F_2 and F_3 generations.

TABLE 10.—*Reaction to stem rust and black chaff in the field in F_2 plants and F_3 lines of Hope×Marquillo and Hope×Supreme wheat crosses*

HOPE × MARQUILLO, F_2 PLANTS [a]

Reaction to stem rust	Number of plants or lines showing indicated reactionto black chaff			
	H	*M*	*L*	Total
R	15	57	38	110
SR	0	22	192	214
S	0	4	108	112
Total	15	83	338	436

HOPE × SUPREME, F_2 PLANTS [a]

	H and *M*	*L*	*L*	Total
R	66	162	----------	228
SR	39	571	----------	610
S	1	359	----------	360
Total	106	1,092	----------	1,198

HOPE × MARQUILLO, F_3 LINES [b]

	H	*L*–	*L*	Total
R	5	18	10	33
R–	2	31	40	73
H	2	28	37	67
S–	1	7	14	22
S	1	1	5	7
Total	11	85	106	202

HOPE × SUPREME, F_3 LINES [c]

	H and *L*–	*L*	*L*	Total
R	5	1	----------	6
R–	11	18	----------	29
H	16	20	----------	36
S–	7	18	----------	25
S	0	7	----------	7
Total	39	64	----------	103

[a] P=less than 0.01. [b] P=0.04. [c] P=0.03.

There was a tendency to linkage in the crosses in the same direction as in the parents, both F_2 plants and F_3 lines resistant to stem rust being more susceptible to black chaff than the rust-susceptible plants and lines. In spite of this linkage, a considerable number of plants and lines appeared to be resistant both to stem rust and

black chaff, and it would seem, therefore, that when Hope is used as one of the parents lines may be obtained from these two crosses that will be resistant to both diseases.

STOMATAL BEHAVIOR IN RESISTANT AND SUSCEPTIBLE WHEAT HYBRIDS

As previously mentioned, Hart (*21*, *22*) has suggested that the "mature plant resistance" of certain wheat varieties and hybrids to stem rust under field conditions may be due to "functional resistance", or the behavior of their stomata, but Peterson (*36*) was unable to verify her observations.

In the present study of stomatal behavior, four F_4 lines of each of the classes—resistant, semiresistant, and susceptible—from the three crosses previously described were selected and grown in 1931, together with the parents. These were space-planted 25 seeds per row in 6-foot rows. The rows were arranged at random for each cross and were examined without knowledge of their identity. The microscopic observations were begun shortly before heading and ended approximately 2 weeks after the heading date of the latest variety or hybrid lines. The first observations were made 30 minutes after sunrise and repeated at 30-minute intervals until the stomata were classed as open. Only the stomata on the leaves were studied. They were examined under the low power of a microscope, without the use of the fine adjustment, while the leaves were on the plant. The rows were always examined in the same order, that is, from 1 to 10, 11 to 20, and 21 to 30. It required 9 minutes to examine a series of 10 rows. The stomata of the leaves on the last row examined may have been more widely open that those of the first row because of the extra 9 minutes of sunlight. Six leaves of approximately the same age were examined in each row. Only one cross and its parents were examined in a day, and the three crosses were taken in a definite rotation on each succeeding day. The Hope×Marquillo hybrids and parents were examined on the first day, Hope×Supreme on the second, Marquillo×Supreme on the third, etc.

The degrees of stomatal opening were classed as open, half open, narrow slits, and closed. It is more difficult to classify the stomata in the two classes half open and open, as the stomata pass gradually from the closed condition to open; hence the narrow-slit class is the most reliable indication of the comparative degree of opening. A study also was made of the number of stomata in each variety and hybrid line. For this purpose three counts of the number in the microscopic field were made at random on each plot.

Table 11 shows the average date of heading, the average number of stomata, and the average number of 30-minute periods required for a variety or hybrid line to attain a certain degree of stomatal opening. There are three very important factors—light, temperature, and humidity—that cannot be controlled under field conditions. Light intensity appears to be the main factor governing stomatal behavior. Cloudiness affects the rate of opening on any particular day and the time required for the stomata to attain a certain degree of opening on the different days. However, it should be possible to detect stomatal differences in the field if stomatal behavior is the basis of functional resistance or susceptibility, although it would be more desirable to make the study where the various factors could be controlled.

TABLE 11.—*Date of heading, number of stomata, and number of 30-minute periods required for stomata to open in parent and F_4 lines of Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme wheat crosses*

[Data represent averages of observed values]

Variety or cross	Number of 30-minute periods required for indicated degree of stomatal opening			Date of heading (1931)	Number of stomata
	Narrow slits	Half open	Open		
Hope (resistant)	2.6	3.7	4.8	July 10	142
Marquillo (semiresistant)	2.6	3.8	4.9	July 4	131
Supreme (susceptible)	2.6	3.8	4.9	July 7	149
Hope×Marquillo F_4 lines:					
Resistant	2.6	3.7	4.7	July 8	147
Do	2.7	3.9	5.0	do	134
Do	2.6	3.9	4.9	July 6	132
Do	2.6	3.9	5.0	July 12	149
Average	2.6	3.9	4.9		
Susceptible	2.6	3.9	5.0	July 8	115
Do	2.7	3.9	5.0	July 6	133
Do	2.5	3.9	4.9	July 10	141
Do	2.6	4.1	5.1	July 4	142
Average	2.6	4.0	5.0		
Hope×Supreme F_4 lines:					
Resistant	2.6	3.7	4.9	July 10	164
Do	3.0	3.9	5.1	July 11	174
Do	2.6	3.7	4.7	July 8	181
Do	2.7	3.7	5.0	July 10	136
Average	2.7	3.8	4.9		
Susceptible	2.9	3.9	5.0	July 8	134
Do	2.9	3.9	5.1	July 10	192
Do	2.7	3.7	5.0	do	142
Do	2.6	3.7	4.9	July 5	152
Average	2.8	3.8	5.0		
Marquillo×Supreme F_4 lines:					
Semiresistant	2.6	3.6	4.9	July 9	146
Do	2.7	4.0	5.1	July 8	131
Do	2.7	4.0	5.1	do	146
Do	2.6	3.7	4.9	July 9	146
Average	2.7	3.8	5.0		
Susceptible	2.6	3.9	5.0	July 4	138
Do	2.5	3.7	5.0	July 8	171
Do	2.6	3.7	4.9	July 9	130
Do	2.6	3.7	4.9	July 6	161
Average	2.6	3.8	5.0		

The data do not indicate any relation between stomatal behavior and rust reaction in any of the three parents or crosses. Since the resistant lines had no rust, the semiresistant lines a little rust, and the susceptible lines much rust, the differences in stomatal behavior, if any, should also have been consistent and distinct.

Climatic conditions were very abnormal during the period of this study. Temperatures were extremely high and moisture was deficient. As the plants were shorter than they normally are, the flag leaves caught the first rays of the morning sun. This may account in part for the regular opening of the stomata on the different varieties and hybrid lines.

One of the difficulties encountered in this study was the differences in the stage of development due to different dates of heading, as indicated in table 11. Hope headed on July 10, Marquillo on July 4, and the Hope×Marquillo lines varied from July 4 to July 12, extending 2 days past the date of the late-heading parent. Supreme headed on July 7, while the hybrid lines with Hope as the other parent headed from July 5 to 11. The heading dates of the Marquillo×Supreme hybrids ranged from July 4 to 9, most of them heading later than either parent. However, there does not appear to be any correlation between the date of heading and the time of opening of the stomata; it therefore appears that the differences in heading dates do not invalidate the conclusions.

The average number of stomata in the microscopic field was 142 for Hope, 131 for Marquillo, and 149 for Supreme. In the Hope×Marquillo hybrids the average number of stomata ranged from 115 to 149; in the Hope×Supreme hybrids, from 134 to 192; and in the Marquillo ×Supreme hybrids, from 130 to 171. There appears to be no relationship between the average number of stomata in a particular line and its resistance or susceptibility to stem rust. These results are in agreement with those obtained by Hursh (*29*).

SUMMARY

Studies were made to determine the manner of inheritance of reaction to stem rust, bunt, and black chaff and of awnedness and color of coleoptile in triangular crosses of the three spring-wheat varieties Hope, Marquillo, and Supreme. A study was made of the inheritance of each individual character and of the independence of different combinations of characters. The relationship between stomatal behavior and mature-plant reaction to stem rust in the field was also studied on the parents and F_4 lines of the triangular crosses.

Epidemic conditions of stem rust were obtained by increasing individual physiologic forms of the rust in the greenhouse and hypodermically inoculating at heading time the plants of the susceptible wheat varieties in the borders and alleys in the field with a water suspension from a mixture of urediospores of all available forms of the rust. In addition, susceptible varieties of wheat were grown in the greenhouse to the heading stage, inoculated with all the available physiologic forms of stem rust, and then transplanted to the field. Bunt infection was obtained by dusting the grain with chlamydospores before sowing. Black-chaff infection was studied under natural infection conditions in the rust nurseries.

In the Hope×Marquillo cross, inheritance of mature-plant reaction to stem rust appeared to depend on three or more factors. Inheritance of the mature-plant semiresistance of Marquillo appeared to depend on factors that were not allelomorphic to those controlling inheritance of the mature-plant resistance of the Hope type. In the Hope×Supreme cross, inheritance of the mature-plant resistance to stem rust appeared to depend on at least two factors. In the Marquillo×Supreme cross, at least three genetic factors appeared to be concerned in the inheritance of mature-plant resistance and susceptibility to stem rust.

Seedlings of the F_3 lines of the Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme crosses were inoculated in the greenhouse with physiologic form 36 of stem rust. The F_3 lines of Hope×Mar-

quillo and Hope×Supreme segregated approximately in the ratio of 1 resistant to 2 segregating to 1 susceptible. All the F_3 lines of Marquillo×Supreme were susceptible.

It was impossible to determine the number of genetic factors involved in the inheritance of reaction to bunt of the three crosses studied, but the results indicate that the greater the susceptibility of one or both parents the greater the tendency toward susceptibility of the hybrids.

Black-chaff infection was light. In general, segregation occurred in the two crosses having Hope as one of the parents, but no conclusion could be reached concerning the number of factors involved in the inheritance of this character. Segregation did not occur in the Marquillo×Supreme cross.

Hope is awned, whereas Marquillo is awnleted. Segregation of this character indicates that a single genetic factor is involved. In the Hope×Supreme cross, in which the Supreme parent is awnless, segregation indicates that two genetic factors are involved. In the Marquillo×Supreme cross one genetic factor appeared to explain the segregation.

Seedlings of Hope have a purple coleoptile in the greenhouse, whereas those of Marquillo and Supreme have a green coleoptile. Segregation of this character in the two crosses having Hope as one of the parents indicates that a single factor is involved. Marquillo and Supreme have distinctly pale-green coleoptiles. In the Marquillo×Supreme cross all plants of the F_3 lines were green.

Independence of reaction to all combinations of stem rust, bunt, and black chaff, and to awn development, and to coleoptile color was studied by means of the χ^2 test.

The inheritance of the following combinations of characters appeared to be independent: Mature-plant reaction to stem rust in relation to bunt, seedling reaction (except in Hope×Marquillo), awnedness, and color of coleoptile; black chaff in relation to bunt, seedling reaction, awnedness, and color of coleoptile; bunt in relation to seedling reaction, awnedness, and color of coleoptile; awnedness in relation to seedling reaction and color of coleoptile; and seedling reaction in relation to color of coleoptile.

There appeared to be a tendency to linkage or association of mature-plant reaction to stem-rust and seedling reaction in the Hope×Marquillo cross and mature-plant reaction to stem rust and reaction to black chaff in the crosses of Hope with Marquillo and Supreme.

The relationship between stomatal behavior and reaction to stem rust was studied microscopically under field conditions. The tests included resistant, semiresistant, and susceptible hybrids from the three crosses Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme. Only slight differences were noted in the stomatal behavior in 1931 and these did not appear to be correlated with rust reaction. It therefore would appear that the resistance of Hope is not due to stomatal behavior.

LITERATURE CITED

(1) AAMODT, O. S.
1923. THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT. Jour. Agr. Research 24: 457–470, illus.

(2) ———
1927. BREEDING WHEAT FOR RESISTANCE TO PHYSIOLOGIC FORMS OF STEM RUST. Jour. Amer. Soc. Agron. 19: 206–218.

(3) ———
1931. VARIETAL TRIALS, PHYSIOLOGIC SPECIALIZATION, AND BREEDING SPRING WHEATS FOR RESISTANCE TO TILLETIA TRITICI AND T. LEVIS. Canad. Jour. Research 5: 501–528.

(4) BIFFEN, R. H.
1905. MENDEL'S LAWS OF INHERITANCE AND WHEAT BREEDING. Jour. Agr. Sci. [England] 1: [4]–48, illus.

(5) BRIGGS, F. N.
1926. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI (BJERK.) WINTER, IN WHEAT. Jour. Agr. Research 32: 973–990, illus.

(6) ———
1930. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN WHITE ODESSA WHEAT. Jour. Agr. Research 40: 353–359, illus.

(7) ———
1930. INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, TILLETIA TRITICI, IN HUSSAR WHEAT. Jour. Agr. Research 40: 225–232, illus.

(8) ———
1931. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF WHITE FEDERATION AND BANNER BERKELEY WHEATS. Jour. Agr. Research 42: 307–313, illus.

(9) CLARK, J. A.
1924. SEGREGATION AND CORRELATED INHERITANCE IN CROSSES BETWEEN KOTA AND HARD FEDERATION WHEATS FOR RUST AND DROUGHT RESISTANCE. Jour. Agr. Research 29: 1–47, illus.

(10) ——— FLORELL, V. H., and HOOKER, J. R.
1928. INHERITANCE OF AWNEDNESS, YIELD, AND QUALITY IN CROSSES BETWEEN BOBS, HARD FEDERATION, AND PROPO WHEATS AT DAVIS, CALIF. U.S. Dept. Agr. Tech. Bull. 39, 40 pp., illus.

(11) FARRER, W.
1901. RESULTS OF THE LAMBRIGG BUNT EXPERIMENTS OF 1900. Agr. Gaz. N.S. Wales 12: 419–430.

(12) ———
1904. REPORT OF THE WHEAT EXPERIMENTALIST. Agr. Gaz. N.S. Wales 15: 1047–1050.

(13) ———
1905. SOME NOTES FOR WHEAT-GROWERS. Agr. Gaz. N.S. Wales 16: 462–472.

(14) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.

(15) GAINES, E. F.
1923. GENETICS OF BUNT RESISTANCE IN WHEAT. Jour. Agr. Research 23: 445–480, illus.

(16) ——— and SINGLETON, H. P.
1926. GENETICS OF MARQUIS×TURKEY WHEAT IN RESPECT TO BUNT RESISTANCE, WINTER HABIT, AND AWNLESSNESS. Jour. Agr. Research 32: 165–181, illus.

(17) GOULDEN, C. H.
1929. BREEDING RUST RESISTANT VARIETIES OF WHEAT—FUNDAMENTAL ASPECTS OF THE PROBLEM. Sci. Agr. 10: 258–267, illus.

(18) ——— NEATBY, K. W., and WELSH, J. N.
1928. THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS TRITICI IN A CROSS BETWEEN TWO VARIETIES OF TRITICUM VULGARE. Phytopathology 18: 631–658, illus.

(19) ——— NEWTON, M., and BROWN, A. M.
1930. THE REACTION OF WHEAT VARIETIES AT 2 STAGES OF MATURITY TO 16 PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Sci. Agr. 11: 9–25, illus.

(20) HARRINGTON, J. B.
1925. THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT. Sci. Agr. 5: 265–288, illus.

(21) HART, H.
1929. RELATION OF STOMATAL BEHAVIOR TO STEM-RUST RESISTANCE IN WHEAT. Jour. Agr. Research 39: 929–948, illus.

(22) ———
1931. MORPHOLOGIC AND PHYSIOLOGIC STUDIES ON STEM-RUST RESISTANCE IN CEREALS. U.S. Dept. Agr. Tech. Bull. 266, 76 pp., illus.

(23) HAYES, H. K.
1929. BREEDING DISEASE RESISTANT VARIETIES OF SMALL GRAINS IN MINNESOTA. Leopoldina 4: 250–262.

(24) ——— and AAMODT, O. S.
1923. A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS. Jour. Agr. Research 24: 997–1012, illus.

(25) ——— PARKER, J. H., and KURTZWEIL, C.
1920. GENETICS OF RUST RESISTANCE IN CROSSES OF VARIETIES OF TRITICUM VULGARE WITH VARIETIES OF T. DURUM AND T. DICOCCUM. Jour. Agr. Research 19: 523–542, illus.

(26) ——— and STAKMAN, E. C.
1922. WHEAT STEM RUST FROM THE STANDPOINT OF PLANT BREEDING. West. Canad. Soc. Agron. Proc. Ann. Meeting (1921) 2: 22–35, illus.

(27) ——— STAKMAN, E. C., and AAMODT, O. S.
1925. INHERITANCE IN WHEAT OF RESISTANCE TO BLACK STEM RUST. Phytopathology 15: [371]–387, illus.

(28) HOWARD, A., and HOWARD, G. L. C.
1915. ON THE INHERITANCE OF SOME CHARACTERS IN WHEAT. II. India Dept. Agr. Mem., Bot. Ser. 7: [273]–285, illus.

(29) HURSH, C. R.
1924. MORPHOLOGIC AND PHYSIOLOGIC STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN. Jour. Agr. Research 27: 381–412, illus.

(30) KIRK, L. E., and IMMER, F. R.
1928. APPLICATION OF THE GOODNESS OF FIT TESTS TO MENDELIAN CLASS FREQUENCIES. Sci. Agr. 8: 745–750.

(31) MCFADDEN, E. S.
1930. A SUCCESSFUL TRANSFER OF EMMER CHARACTERS TO VULGARE WHEAT. Jour. Amer. Soc. Agron. 22: 1020–1034.

(32) MELCHERS, L. E., and PARKER, J. H.
1922. INHERITANCE OF RESISTANCE TO BLACK STEM RUST IN CROSSES BETWEEN VARIETIES OF COMMON WHEAT (TRITICUM VULGARE). (Abstract) Phytopathology 12: 31–32.

(33) NEATBY, K. W.
1931. FACTOR RELATIONS IN WHEAT FOR RESISTANCE TO GROUPS OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Sci. Agr. 12: 130–154, illus.

(34) ——— and GOULDEN, C. H.
1930. II. THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS TRITICI IN CROSSES BETWEEN VARIETIES OF TRITICUM VULGARE. Sci. Agr. 10: 389–404, illus.

(35) PERCIVAL, J.
1921. THE WHEAT PLANT; A MONOGRAPH. 463 pp., illus. London.

(36) PETERSON, R. F.
1931. STOMATAL BEHAVIOR IN RELATION TO THE BREEDING OF WHEAT RESISTANCE TO STEM RUST. Sci. Agr. 12: 155–173, illus.

(37) POWERS, L.
1932. CYTOLOGIC AND GENETIC STUDIES OF VARIABILITY OF STRAINS OF WHEAT DERIVED FROM INTERSPECIFIC CROSSES. Jour. Agr. Research 44: 797–831, illus.

(38) PYE, H.
1909. WHEAT IMPROVEMENT COMMITTEE. DISEASES AND PESTS OF CEREALS. Jour. Dept. Agr. Victoria 7: 368–373.

(39) QUISENBERRY, K. S.
1931. INHERITANCE OF WINTER HARDINESS, GROWTH HABIT, AND STEM-RUST REACTION IN CROSSES BETWEEN MINHARDI WINTER AND H-44 SPRING WHEATS. U.S. Dept. Agr. Tech. Bull. 218, 46 pp., illus.
(40) STAKMAN, E. C.
1914. A STUDY OF CEREAL RUSTS: PHYSIOLOGIC RACES. Minn. Agr. Expt. Sta. Bull. 138, 56 pp., illus.
(41) STEWART, G., and HEYWOOD, D. E.
1929. CORRELATED INHERITANCE IN A WHEAT CROSS BETWEEN FEDERATION AND A HYBRID OF SEVIER × DICKLOW. Jour. Agr. Research 39: 367–392, illus.
(42) ——— and TINGEY, D. C.
1928. TRANSGRESSIVE AND NORMAL SEGREGATIONS IN A CROSS OF MARQUIS × FEDERATION WHEATS. Jour. Amer. Soc. Agron. 20: 620–634, illus.
(43) WALDRON, L. R.
1929. THE RELATIONSHIP OF BLACK CHAFF DISEASE OF WHEAT TO CERTAIN PHYSICAL AND PATHOLOGICAL CHARACTERS. Science (n.s.) 70: 268.

CORRELATED INHERITANCE OF REACTION TO STEM RUST, LEAF RUST, BUNT, AND BLACK CHAFF IN SPRING-WHEAT CROSSES [1]

By H. K. HAYES, *chief, Division of Agronomy and Plant Genetics, Minnesota Agricultural Experiment Station, and collaborator, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture;* E. R. AUSEMUS, *associate agronomist, Division of Cereal Crops and Diseases;* E. C. STAKMAN, *head of section of plant pathology, Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station, and agent, Bureau of Plant Industry;* and R. H. BAMBERG, *agent, Division of Cereal Crops and Diseases* [2]

INTRODUCTION

For more than 20 years cooperative studies to develop rust-resistant varieties of wheat have been under way between the Bureau of Plant Industry of the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. In recent years the objective has been to breed high-yielding, high-quality varieties of wheat resistant not only to stem rust (*Puccinia graminis* Pers.) but to other important diseases, such as leaf rust (*P. triticina* Eriks.), bunt (*Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn), scab (*Gibberella saubinetii* (Mont.) Sacc.), black chaff (*Bacterium translucens undulosum* Smith, Jones, and Reddy), and root rots, principally those caused by *Helminthosporium sativum* Pam., King, and Bak., and *Fusarium* spp.

Hope and H-44, the varieties used most recently as the parents resistant to stem rust, leaf rust, and bunt, are very susceptible to the bacterial black-chaff disease. Observation and certain experiments indicate that there is some tendency for resistance to stem rust and susceptibility to black chaff to be associated in the segregates of crosses having as one of the parents either Hope or H-44 or a related strain. The experiments reported in this paper were conducted to ascertain the extent of genetic linkage or association of these diseases. It is thought that the results may supplement in a useful way a companion contribution by one of the writers.[3]

Since most of the pertinent literature has been reviewed in the companion paper referred to, it seems unnecessary to present such a review here. An abstract of a paper presented at the Sixth International Congress of Genetics at Ithaca, N.Y., in 1932, gives a brief outline of the studies in Minnesota that have led to the present appreciation of the importance of mature-plant resistance.[4]

[1] Received for publication Oct. 11, 1933; issued February 1934. Cooperative investigations of the Minnesota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture. Paper no. 1093 of the Journal series, Minnesota Agricultural Experiment Station.

[2] The writers acknowledge the assistance of C. C. Allison, of the Division of Cereal Crops and Diseases, in taking notes in 1930.

[3] AUSEMUS, E. R. CORRELATED INHERITANCE OF REACTION TO DISEASES AND OF CERTAIN BOTANICAL CHARACTERS IN TRIANGULAR WHEAT CROSSES. Jour. Agr. Research 48: 31-57, illus. 1934.

[4] HAYES, H. K. THE GENETICS OF RUST RESISTANCE IN WHEAT. (Abstract.) Sixth Internatl. Cong. Genetics, Ithaca, Proc. 2: 81-83. 1932.

MATERIALS AND METHODS

The experiments reported herein were conducted in 1929 and 1930 at University Farm, St. Paul, Minn. Those for the first season were limited to a cross between H-35 and Marquis and those for the second season to crosses in which H-44 was used as one parent and Kota × Marquis No. II-19-167 or Double Cross No. II-21-28 as the other. H-44 and H-35 were obtained from crosses between emmer and Marquis wheat. They have 21 chromosomes, and so far have been highly resistant to stem rust under field conditions. H-44 has been resistant to leaf rust and also to bunt. Both are very susceptible to black chaff.

Kota × Marquis No. II-19-167 and Double Cross No. II-21-28 were selected as parents on the basis of preliminary yield experiments and milling and baking tests. They appear to excel in agronomic characters and in milling and baking quality. They are moderately resistant to stem rust, highly resistant to black chaff, susceptible to leaf rust, and moderately susceptible to bunt.

The parents and F_3 lines were grown in special nurseries, the plants being spaced 3 inches apart in rows 1 foot apart.

The F_3 lines were from a random selection of F_2 plants. Approximately 10 rows of each parent were grown at regular intervals within each series of F_3 lines. One or two rows of each line, each row containing 25 seeds, were sown in blocks 6 by 132 feet, thus allowing 132 rows to a block. The blocks were surrounded by rows of susceptible varieties sown crosswise through the alleys and in the border rows. A rust epidemic was induced by dusting urediospores on plants in the heading stage in the greenhouse and then transplanting these inoculated plants to the border and alley rows in the rust nursery. In addition, a water suspension of urediospores of the available forms of stem rust was injected hypodermically into the upper leaves of plants in the heading stage in the border and alley rows. This method has been very satisfactory.

Bunt was studied in a separate nursery; black chaff was studied in the rust nursery. For the bunt studies the wheat was inoculated by dusting with bunt spores just before sowing. Black chaff developed from natural infection.

RUST

In order to compare the reaction of the various F_3 lines to stem rust and leaf rust the plants of each F_3 line were placed in one of three classes, designated *R*, *SR*, and *S*. *R* includes plants with no infection or only a trace of rust of the type of pustule commonly associated with resistance; *SR*, or semiresistant, includes plants that appeared rather resistant, the type of pustule being smaller than in the susceptible group; and *S* includes susceptible plants on which there were large confluent pustules and severe infection. Each F_3 line was classified on the basis of reaction of all F_3 plants. The lines were then grouped according to breeding behavior. The groups for reaction to leaf rust and stem rust were as follows:

R = Breeding true for *R*.
R— = Containing *R* and *SR* plants, or breeding true for *SR* type.
H = Segregating for *R*, *SR*, and *S*.
H— = Segregating for *SR* and *S*.
S = Breeding true for *S*.

BUNT

The number of bunted spikes was divided by the total number of spikes in each line, the result showing the percentage of infection. The counts were made separately for each parental and F_3 line.

BLACK CHAFF

Notes on black chaff were taken on individual plants and the degree of infection was indicated as heavy, medium, or light. The lines were then grouped into 7 classes, as follows: Class 1, very heavily infected and apparently homozygous for susceptibility; classes 2 to 6, intermediate degrees of reaction; classes 3, 4, and 5, apparently segregating and containing both highly susceptible and apparently very resistant plants; class 7, highly resistant and showing only slight traces of black chaff.

EXPERIMENTAL RESULTS

The results of the experiments on reaction to individual diseases are presented first and are followed by a study of independence in reaction to the various combinations of diseases.

REACTION TO STEM RUST

The reaction to stem rust is summarized in table 1. Comparisons are shown between the reaction of the F_3 lines from the crosses of H-44 with Kota×Marquis No. II-19-167 and with Double Cross No. II-21-28 and the reaction of the parent rows, in 1930, and the reaction of the progeny of F_3 plants in the F_4 generation, in 1931. The purpose of the F_4 test was to determine the accuracy of the F_3 classification.

TABLE 1.—*Stem-rust reaction in parent rows and F_3 and F_4 lines of three wheat crosses grown at University Farm, St. Paul, Minn., 1929, 1930, and 1931*

Parent or generation	Year	Parent-plant reaction	Number of rows or lines showing indicated reaction					Total
			R	*R*−	*II*	*II*−	*S*	
H-44 rows	1930		8	2				10
Kota × Marquis No. II-19-167 do	1930			6		3	1	10
F_3 lines	1930		35	78	27	25	2	167
H-44 rows	1931	*R*	2					2
Kota × Marquis No. II-19-167 do	1931	*S*		1	1			2
F_4 lines	1931	*R*	8	1	7	2		18
Do do	1931	*SR*		3	5	9	12	29
Do do	1931	*S*				1	3	4
H-44 rows	1930		14	1				15
Double Cross No. II-21-28 do	1930		1	12	1	1		15
F_3 lines	1930		35	54	68	26	4	187
H-44 rows	1931	*R*	3					3
Double Cross No. II-21-28 do	1931	*R*		2				2
Do do	1931	*SR*		1				1
F_4 lines	1931	*R*	26	9	12	3	2	52
Do do	1931	*SR*	3	13	5	3	10	34
Do do	1931	*S*				2	7	9
H-35 rows	1929		6					6
Marquis do	1929						5	5
F_3 lines	1929		12	16	69	14	16	127

In the studies of the cross between H-44 and No. II-19-167 grown in 1930, all 10 rows of the H-44 parent were classed in the groups *R* and *R*−. Of the 10 parent rows of Kota×Marquis No. II-19-167, 6 were placed in the *R*− group, whereas 4 rows gave a preponderance of

susceptible plants. This might be explained by the presence of physiologic forms that caused the epidemic in particular rows, or by environmental conditions in the field that caused more severe infection in some rows than in others. Of 167 F_3 lines selected at random, 35 were placed in the resistant group. If the correct classification of the *R* and *R*− lines is in a ratio of 4 to 1, as in H-44, 8 or 9 more lines might be considered as breeding true for resistance, making a total of approximately 44 out of 167 for this group. This is a ratio of about 1 homozygous resistant to 3 susceptible. These results may be explained on the basis of a single-factor difference for the H-44 type of mature-plant resistance.

Two rows of each of the parents H-44 and No. II-19-167 were grown in the rust nursery in 1931. Both rows of H-44 were placed in the *R* class, whereas the progeny of two plants of No. II-19-167, classed in 1930 as *S*, were placed in *R*− in one case and in *H* in the other. Various categories of F_3 plants were studied in F_4, the selections being made from segregating F_3 lines. The progeny of *R* plants in 1931 produced homozygous *R* lines and lines classed in all other groups except the *S* group. The *SR* plants produced no lines that bred true for the *R* group and only 3 for the *R*− group; other lines were classed in the *H*, *H*−, and *S* groups. One of the progenies of the 4 susceptible plants was placed in the *H*− class and 3 in the *S* class.

An analysis was made also of the data from the cross of H-44 with Double Cross No. II-21-28. Apparently one factor may explain the H-44 type of reaction in this cross. While no attempt has been made in these studies to determine the number or nature of factors contributed by the Double Cross parent, the possible relation of such factors to those governing the mature-plant resistance of H-44 has been considered.

In the cross H-35×Marquis it is impossible to say how many factors are involved for the H-35 type of reaction. Considering the proportion of heterozygous lines it seems probable that the results cannot be explained satisfactorily on a single-factor basis. Studies made previously in Minnesota and at the Dominion Rust Laboratory at Winnipeg, Manitoba, indicate that several genetic factors are responsible for the mature-plant resistance of the Kota type and of the Minnesota double crosses.

REACTION TO LEAF RUST

Data on the reaction to leaf rust of the parents and the F_3 lines of two wheat crosses are presented in table 2. From these data it is apparent that segregation has occurred. It appears rather easy to recover the type of resistance of the H-44 parent. No definite conclusion can be made regarding the number of genetic factors involved. If the two crosses are considered together, it is seen that, of 354 F_3 lines grown from random selections of F_2 plants, 28 lines were grouped as *R* and 47 lines as *R*−. It appears that only one or two factor pairs are necessary to explain the results. Little is known of the causes of mature-plant resistance to leaf rust or of the part played by physiologic forms in producing the results reported in this paper.

TABLE 2.—*Leaf-rust reaction in parent rows and F_3 lines of H-44×(Marquis×Kota No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

Parent or generation	Number of rows or lines showing indicated reaction					
	R	*R*–	*H*	*H*–	*S*	Total
H-44 ..... rows	4	3	2	1		10
Marquis×Kota No. II-19-167 ..... do		1		3	6	10
F_3 ..... lines	11	28	78	22	28	167
H-44 ..... rows	9	5	1			15
Double Cross No. II-21-28 ..... do			2	1	12	15
F_3 ..... lines	17	19	115	16	20	187

REACTION TO BUNT

The data on the reaction to bunt of the parent rows and F_3 lines of two wheat crosses are presented in table 3.

TABLE 3.—*Bunt reaction in parent rows and F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

Parent or cross	Number of rows or lines showing indicated percentage of bunt infection							
	0	0.1–3	3.1–6	6.1–9	9.1–12	12.1–15	15.1–18	Total
H-44 ..... rows	8	1						9
Kota×Marquis No. II-19-167 ..... do		5	3	1		1		10
F_3 ..... lines	79	55	17	7	5	1	2	166
H-44 ..... rows	14	1						15
Double Cross No. II-21-28 ..... do	2	4	4	3	2			15
F_3 ..... lines	121	45	11	6	3	1		187

H-44 appears to be highly resistant. Of the 24 rows grown, 22 had no bunt and 2 had only 0.1 to 3 percent. From the 2 crosses 353 lines were grown, and of these 200 had no bunt. The bunt infection was light in the parents as well as in the F_3 lines, and as 2 of the 15 lines of No. II-21-28, showed no bunt, it seems impossible to reach any conclusion regarding the inheritance of bunt reaction.

REACTION TO BLACK CHAFF

Little is known about the conditions necessary for black-chaff infection, and consequently it is difficult to obtain a satisfactory epidemic under field conditions. Much variability in infection is found in different parts of the same nursery or in fields relatively close together. An attempt was made to induce an epidemic in the cereal pathology garden, but a more satisfactory epidemic was obtained without artificial means in the stem-rust and leaf-rust nurseries. The data obtained under these conditions are presented in table 4.

TABLE 4.—*Reaction to black chaff in parent rows and F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown in 1930, and of H-35×Marquis, grown in 1929, at University Farm, St. Paul, Minn.*

Parent or generation	Year	Number of rows or lines included in indicated black-chaff class							Total
		1	2	3	4	5	6	7	
H-44 rows	1930			3		5	1	1	10
Kota × Marquis No. II-19-167 do						1		9	10
F_3 lines			1	5	4	69	19	69	167
H-44 rows		1	1	2	4	5	0	2	15
Double Cross No. II-21-28 do							1	14	15
F_3 lines		1	19	15	45	41	19	47	187
H-35 rows	1929		3	1	2				6
Marquis do						1	1	4	6
F_3 lines			5		93	23	3	3	127

The variable infection of H-44 in 1930 emphasizes the difficulty of the study. Thus, of 25 rows 3 showing only a trace of infection were placed in class 7, although it is known that in general H-44 is highly susceptible. It is apparent that segregation occurred in F_2 and that a large percentage of the F_3 lines was as free from infection as Kota×Marquis No. II-19-167 and Double Cross No. II-21-28, the resistant parents.

Infection was more severe in 1929, when the cross H-35×Marquis was grown. Several F_3 lines appeared to be resistant and were infected not more than was Marquis.

INDEPENDENCE OF REACTION TO THE FOUR DISEASES

In an attempt to synthesize hybrids resistant to two or more diseases it is important to determine whether there is association or independence in inheritance. If there is association and if the association is due to physiologic causes and is dependent upon the same genotypic basis, it would be impossible to obtain a new combination or relationship of the characters. If the association is dependent upon genetic linkage a new combination of parental characters would depend upon the closeness of such linkage; that is, upon the frequency of crossing-over of the genes concerned.

Table 5 shows the crosses studied, the number of F_3 lines, the character pairs studied, χ^2 for independence, n (degrees of freedom), and P. It is apparent that reaction to the following pairs of diseases is inherited independently or that the linkage relation is very loose: Stem rust and bunt; leaf rust and bunt; leaf rust and black chaff; black chaff and bunt. Stem rust and leaf rust appear to be distinctly associated. In the two comparisons of the reaction to stem rust and leaf rust, the values of P for the two crosses with H-44 were 0.03 and 0.05. In the comparison of the reaction to black chaff and stem rust the values of P were 0.05, less than 0.01, and 0.11. These were the only P values obtained that indicated a linkage in inheritance.

It is interesting to observe the contingency surfaces for those cases in which there appeared to be an association for reaction to two diseases. In the crosses in which H-44 was used as one parent, the relationship for reaction to stem rust and black chaff is shown in table 6 and that for stem rust and leaf rust in table 7.

TABLE 5.—*Independent inheritance of reaction to various diseases in the F_3 lines of wheat crosses grown at University Farm, St. Paul, Minn., 1930*

Cross	Number of F_3 lines	Characters compared	χ^2	n	P
H-44×Kota×Marquis No. II-19-167	167	Stem rust and leaf rust	18.56	9	0.03
H-44×Double Cross No. II-21-28	187	do	12.81	6	.05
H-44×Kota×Marquis No. II-19-167	166	Stem rust and percentage of bunt	11.85	9	.22
H-44×Double Cross No. II-21-28	187	do	7.52	6	.27
H-44×Kota×Marquis No. II-19-167	167	Stem rust and black chaff	16.81	9	.05
H-44×Double Cross No. II-21-28	187	do	41.53	9	(a)
H-35×Marquis	127	do	4.45	2	.11
H-44×Kota×Marquis No. II-19-167	166	Leaf rust and percentage of bunt	7.26	9	.61
H-44×Double Cross No. II-21-28	187	do	7.15	4	.09
H-44×Kota×Marquis No. II-19-167	167	Leaf rust and black chaff	10.29	9	.33
H-44×Double Cross No. II-21-28	187	do	6.60	6	.37
H-44×Kota×Marquis No. II-19-167	166	Black chaff and percentage of bunt	3.37	9	.50
H-44×Double Cross No. II-21-28	187	do	8.09	6	.24

[a] Less than 0.01.

TABLE 6.—*Reaction to stem rust and black chaff in F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

H-44×(KOTA×MARQUIS NO. II-19-167) [a]

Black-chaff class	Number of F_3 lines showing indicated reaction to stem rust				Total
	R	*R*−	*H*	*H*−, *S*	
2, 3, 4	5	3	2		10
5	16	30	14	9	69
6	5	9	4	1	19
7	9	36	7	17	69
Total	35	78	27	27	167

H-44×DOUBLE CROSS NO. II-21-28 [b]

1, 2, 3	18	5	9	3	35
4	6	15	21	3	45
5	5	10	19	7	41
6, 7	6	24	19	17	66
Total	35	54	68	30	187

[a] P=0.05. [b] P=less than 0.01.

TABLE 7.—*Reaction to stem rust and leaf rust in F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

H-44×KOTA×MARQUIS NO. II-19-167 [a]

Reaction to leaf rust	Number of F_3 lines showing indicated reaction to stem rust				Total
	R	*R*−	*H*	*H*−, *S*	
R, *R*−	9	20	5	5	39
H	21	32	16	9	78
H−	3	7	5	7	22
S	2	19	1	6	28
Total	35	78	27	27	167

H-44×DOUBLE CROSS NO. II-21-28 [b]

R, *R*−	13	9	11	3	36
H	18	30	46	21	115
H−, *S*	4	15	11	6	36
Total	35	54	68	30	187

[a] P=0.03. [b] P=0.05.

It is apparent that linkage is in the same direction as in the parent varieties, that is, there is a preponderance of lines resistant to stem rust, susceptible to black chaff, and resistant to both stem rust and leaf rust. However, in the stem-rust-resistant lines the proportion of lines in black-chaff classes 6 and 7 (resistant) is much greater in the F_3 hybrids than in the H-44 parent, which leads to the belief that lines resistant to the three diseases can be obtained from crosses in which H-44 is used as one parent.

SUMMARY

Correlated reaction in wheat to stem rust, leaf rust, bunt, and black chaff was studied in the F_3 progeny of crosses between H-44 with Double Cross No. II-21-28 and Kota×Marquis No. II-19-167, respectively. The F_3 lines were grown from random selections of F_2 plants.

Epidemics of stem rust, leaf rust, and bunt were created artificially, but black chaff developed naturally in the rust nurseries.

Inheritance of stem-rust resistance of the mature-plant type of the H-44 parent appeared to be dependent upon a single genetic factor difference. The moderate plant resistance of Nos. II-19-167 and II-21-28 appeared to be dependent upon factors not allelomorphic to those determining mature-plant resistance of the H-44 type, as susceptible lines were obtained in the F_3 generation. There was some indication that more than a single-factor pair was necessary to explain the stem-rust resistance of mature plants of the H-35 parent in the crosses with Marquis.

It was impossible to determine the number of factor pairs responsible for segregation of reaction to leaf rust, bunt, and black chaff, although a considerable number of resistant types were obtained in all cases.

Independence of reaction to all combinations of stem rust, leaf rust, bunt, and black chaff was studied by the use of χ^2 for independence.

The inheritance of reaction to the following combinations of diseases appeared to be independent: Stem rust and bunt, leaf rust and bunt, leaf rust and black chaff, and black chaff and bunt.

There appeared to be linkage in the inheritance of reaction to stem rust and leaf rust and of reaction to stem rust and black chaff. However, a comparison of the F_3 reactions with those of the H-44 parent indicates the possibility of combining the mature-plant resistance to stem rust of the H-44 type with resistance to black chaff of many varieties and hybrids of common wheat.

EFFECT OF WASHING ON THE KEEPING QUALITY OF HENS' EGGS [1]

By REECE L. BRYANT, *graduate student, Department of Poultry Husbandry,* and PAUL FRANCIS SHARP, *professor of dairy chemistry, Department of Dairy Industry, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

Under the ordinary system of poultry management, a considerable percentage of the eggs gathered are dirty. Such eggs bring a lower price than naturally clean ones because of their insanitary appearance and poor keeping quality. If the dirty eggs are cleaned by washing, the dirt is removed but the keeping quality is not improved; according to current ideas, it is materially lessened.

The explanation most frequently offered to account for the belief that washed eggs do not keep so well as unwashed clean ones is that washing removes a film from the surface of the eggs which acts as a protective coating. Its removal is supposed to open the pores of the shell, thus permitting the entrance of micro-organisms which cause spoilage, and accelerating the escape of water, which results in shrinkage or loss in weight of the egg during marketing and storage.

The work herein recorded was undertaken to determine the effect on the keeping quality of eggs of washing and of the type of solution used in washing. As an incidental part of the study a few comparisons were made between cleaning by washing and by dry abrasion.

LITERATURE

The general prevalence of dirty eggs is indicated by several investigators.

In an investigation covering 3 years, Huttar (*1*) [2] found that among the eggs produced by the Cornell poultry flock, the percentage of dirty eggs ranged from 9.8 in July to 24.6 in March and averaged 17.7. He reports:

> The flocks from which these eggs were gathered are managed about the way that the average commercial poultryman manages his flock.

Van Wagenen (*32*) studied the effect of nesting material and litter on the production of dirty eggs. He found that the percentage of dirty eggs varied from 77 for the experiment with straw litter and no nesting material to 23.2 with straw litter and shavings for nesting material.

Pennington and Pierce (*20*) examined a large number of eggs in the New York market. In one series of 258,496 dozen they found 12.58 percent dirty and in another series of 238,446 dozen they found 13.40 percent dirty. Perhaps some of the eggs which they classified as clean were actually washed dirty eggs.

[1] Received for publication Mar. 1, 1933; issued February 1934. The major part of this paper was taken from a thesis presented by the senior writer to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy, June 1928.

[2] Reference is made by number (italic) to Literature Cited, p. 87.

Sharp (*26*) examined 30 samples of eggs of the dozen each obtained from lots offered for sale in a city of up-State New York. Using special tests for detecting the cleaned eggs, he found that 39 percent of the eggs were either cleaned or dirty.

Some of the factors which affect the loss in weight of eggs have been studied. In 1908 Cook, in a report by Wiley et al. (*33*), stated that eggs in storage for 7.5 months sustained a loss in weight equivalent to 5.4 percent of the total weight, which is largely water from the whites. Through improved control of storage conditions the loss in weight is now about 3 to 5 percent for a storage period of about 8 months.

Greenlee (*10*) pointed out that water also passes from the white to the yolk. In his experiments he found that the loss in weight may be described by a parabolic curve, the rate of loss increasing with temperature and decreasing with time. Neither Cook nor Greenlee indicate whether they kept their eggs in an environment of constant humidity.

Dunn (*8, p. 55*) stated:

> If we regard the rate at which an egg loses weight as an expression of the permeability of the envelopes which enclose it, and especially the porosity of the shell, then the shells of eggs must be more variable than the sizes of the eggs themselves.

Dunn (*9*) determined the correlation between the fresh weight and the percentage loss in 2 lots of eggs and obtained the values $r = -0.331 \pm 0.040$ and $r = -0.357 \pm 0.068$. When the fresh weight was correlated with the absolute loss, the value was $r = +0.187 \pm 0.044$. The larger the eggs the greater was the absolute loss but the smaller the percentage loss. Considering the rate of evaporation per unit surface, Dunn concluded (*9, p. 170*): "It is legitimate to assume * * * that the shells of larger eggs are somewhat less porous and tend to conserve moisture better than the shells of smaller eggs."

Curtis (*7*) and Jull (*16*) found that the weight of the shell was quite variable as compared with other parts of the egg.

The conditions under which bacteria will grow in egg white and shell eggs have received considerable attention. Wurtz (*34*), Scholl (*24*) Laschtschenko (*17*), Rettger and Sperry (*22*), and others have shown that egg white has germicidial properties. Healy and Peter (*13*) and Sharp and Powell (*27*) reported that the hydroxyl-ion concentration of the white of eggs increases very rapidly after the eggs are laid, owing to the loss of carbon dioxide, and approaches as a limit pH 9.5-9.7. Sharp and Whitaker (*29*) and Stark and Sharp (*30*) demonstrated that the germicidal action of the egg white is influenced markedly by this change in hydroxyl-ion concentration.

Pennington (*19*) and Stiles and Bates (*31*) found that a considerable number of fresh eggs contained bacteria, although the bacterial count in most cases was low. They plated the material as soon as the eggs were opened. Since others were skeptical because this method yielded so many sterile eggs, they separated the white and yolk and incubated the yolk in a flask for some time before plating. Using this method, Maurer (*18*) found bacteria in 22.9 percent of one lot of 292 fresh eggs and in 25.4 percent of another lot of 283 eggs. Bushnell and Maurer (*6*) found that 23.7 percent of 275 eggs examined contained bacteria, and Hadley and Caldwell (*12*) found that of 2,500 fresh eggs examined 8.7 percent contained bacteria. This method of separating the yolk and allowing it to incubate for some time before

plating has the very serious disadvantage that the yolk may become contaminated durings its separation from the egg and introduction into the flask.

The study of the bacteriology of fresh eggs by Rettger (*21*) deserves especial consideration. The eggs which he examined were normal clean ones. After incubating the whole yolk from 3,510 fresh eggs he found 9.5 percent infected. Of 647 eggs in which he pipetted 10 cc of yolk for a sample for incubation, the infection was 3.86 percent. He says: "Even 3.9 percent is, in all probability, considerably above the actual figures, could accidental invasion of bacteria in the tests be entirely prevented."

If bacteria are present in fresh eggs, the number should be increased by incubation. Rettger incubated 1,746 eggs for 7 to 10 days and found 2.75 percent infection; 2,166 eggs for 2 weeks and found 1.3 percent infection; and 1,984 eggs for 3 weeks and found 3.6 percent infection.

The following statements by Rettger are so significant in relation to the problem treated in this paper that they are quoted (*21, pp. 207, 208, 210*):

> It is impossible to find a satisfactory explanation of the unqualified success which is attained with some of the strongly advocated processes for preserving or pickling eggs, unless it is assumed that sound, fresh eggs are, as a rule, sterile. * * *
>
> Hence, the only safeguard of the contents of the egg against bacterial decomposition from within is a state of absolute asepsis or sterility at the outset. * * *
>
> The soiling of shells, especially with fecal matter, lowers the chances of preservation of the contents. Eggs which are soiled are no longer germproof.

Jenkins, Hepburn, Swan, and Sherwood (*14*) reported that they found bacteria in few naturally clean eggs after storage; bacterial infection was greater in stored dirty eggs and greater still in stored washed dirty eggs.

Jenkins and Pennington (*15*) found that dirty eggs showed 12 to 30 bad eggs per case before the candle and 10.5 to 29 additional on breaking the eggs. They stated that it was not always possible to recognize washed eggs.

METHODS

The factor most often used in judging the quality of eggs is the size of the air cell, since it can be seen clearly in the candling test. Since the increase in size of the air cell is due to evaporation of water from the egg, the loss in weight of the egg becomes the most accurately measurable factor in determining the effect of the various treatments on evaporation. The number of pores in the shells was correlated with the loss in weight of the eggs as a possible explanation of the individual variations that were found.

During storage, water passes from the white of the egg to the yolk. and consequently the percentage of total solids in the yolk decreases, The percentage of total solids in the white increases as a result of the passage of water to the air and to the yolk. A determination of the total solids in the white and yolk is, therefore, an aid in determining changes in quality.

The ability of the yolk to stand up in a dish after the egg is broken is a quality factor which is readily expressed numerically by dividing the height by the width. The better the quality of the yolk, the higher the value of this quotient (*28*).

The hydrogen-ion concentration of the white and yolk was determined as an indication of the storage conditions (*25*).

If bacteria grow abundantly in the egg, it deteriorates rapidly in quality. Since in washing eggs (especially dirty ones) bacteria may be rubbed through the pores of the shell, a comparison of the bacteria found in the white and yolk of the eggs subjected to the various treatments was made.

Clean as well as dirty eggs were washed. By washing clean eggs, the influence of the actual washing itself could be studied without the complicating factor of dirt on the shell.

The eggs, after being subjected to the different treatments, were stored either at 35° C. or at room temperature, 20° to 25°. This relatively high temperature was chosen because at higher temperatures deterioration is more rapid and thus the time required for the experiments was shortened.

Since eggs lose water at different rates, depending on the humidity of the air in which they are stored, the eggs were kept in air of constant humidity. This was accomplished by covering the bottom of a closed container with a saturated sodium chloride solution containing an excess of undissolved sodium chloride. The eggs were supported on a wire tray about 3 inches above the surface of the solution. The container was large enough to permit the eggs of an entire experiment to be placed in a layer one egg deep. The humidity at the surface of the solution was approximately 75 percent.

The eggs used were laid by the flocks of the Poultry Department of Cornell University during 1927–28. They were infertile eggs, produced by White Leghorn pullets. The eggs were put through the various treatments and placed in storage the day they were laid. (An exception in the case of the extremely dirty eggs will be noted later.) No less than 6 eggs, and frequently 12, were subjected to each treatment in an experiment. At stated intervals during the storage period, each egg was weighed to the nearest milligram. On the twentieth day they were candled, and the apparent pores or spots which could be seen before the candle were counted on 1 cm^2.

At the end of the storage period, 27 to 30 days, the following factors were determined: (1) Number of pores that gave bubbles when the egg was immersed in water and placed under a vacuum; (2) breaking strength of the shell; (3) presence of bacteria in the white; (4) presence of bacteria in the yolk; (5) height and width of the yolk; (6) total solids in the white; (7) total solids in the yolk; (8) pH value of the white; and (9) pH value of the yolk.

When the eggs were to be cleaned with water or a solution, they were immersed for about a minute in a container sufficiently large to hold both liquid and eggs. Each egg was then scrubbed with a fairly stiff brush, rinsed in tap water, and placed on a rack to dry. The cleaning treatments, selected because of some special property of the solution, such as acidity, alkalinity, bactericidal effect, or abrasiveness, were as follows: (1) Control eggs stored without treatment; (2) eggs washed with distilled water; (3) washed with N/10 sulphuric acid; (4) washed with N/10 sodium hydroxide; (5) washed with a water-glass solution, prepared by diluting 1 volume of commercial sodium silicate, used for preserving eggs, with 12 volumes of water; (6) washed with a soap solution prepared by dissolving 50 g of white naphtha soap in 3 l of water; (7) washed with a fairly strong

solution of Wyandotte alkaline washing powder; (8) soaked in water, dusted with powdered Sapolio, scrubbed with a brush, and rinsed; (9) washed with a hypochlorite solution prepared by dissolving 1 part of a commercial hypochlorite powder in 100 parts of water; (10) cleaned with sandpaper; (11) dipped for 3 seconds (without previous treatment) in oil that had been heated to 125° C.; (12) washed with distilled water and oil-dipped; (13) sandpapered and oil-dipped; (14) washed with soap and oil-dipped; (15) washed with hypochlorite solution and oil-dipped.

EXPERIMENTAL DATA

FACTORS AFFECTING LOSS IN WEIGHT OF EGGS

It was found that the eggs lost about 0.025 g each after being washed and dried in air. Just how much of this loss was due to the removal of material from the surface of the egg, and how much to the evaporation of water during the drying, is not known.

METHOD OF CLEANING

To determine the loss in weight of eggs cleaned by different methods and held at different temperatures, the following experiments were made:

1. Clean eggs, stored in cartons, holding temperature 35° C.—Naturally clean eggs were gathered, washed, dried, and weighed on the day they were laid. They were then placed in cartons holding 1 dozen each, and held for 27 days at 35° C. in a closed container over a saturated solution of sodium chloride. One dozen eggs were subjected to each treatment. Each egg was weighed at 3-day intervals. It was thought that possibly by retarding diffusion, the cartons might have obscured the effect of the washing. Therefore in subsequent experiments the eggs were placed in a layer one egg deep on a woven-wire tray suspended about 3 inches above the surface of the sodium chloride solution.

2. Clean eggs, holding temperature 35° C.—The procedure was the same as in 1, except that the eggs were not stored in cartons and were weighed at 6-day intervals for 30 days.

3. Extremely dirty eggs, holding temperature 35° C.—A compost of hen manure and a bouillon culture of *Pseudomonas pyocyanea* were made up to a pasty consistency. Fresh, clean eggs were dipped in the compost, allowed to dry overnight, and washed the following day. The eggs were made very dirty, so that extreme conditions might be studied. The weighing was discontinued in practically every instance because the gas pressure created by bacterial infection within the egg caused the contents to spew through the pores, resulting in abnormal decreases in weight. Six eggs were subjected to each treatment.

4. Naturally dirtied eggs, holding temperature 35° C.—Eggs very dirty when gathered from the nest were subjected in lots of six eggs each to the different cleaning procedures and then held at 35° for 30 days.

5. Clean eggs, holding temperature 20° to 25° C.—Naturally clean eggs in lots of six eggs each were cleaned as indicated, and stored for 27 days at room temperature, 20° to 25°, over a saturated solution of sodium chloride.

6. Extremely dirty eggs, holding temperature 20° to 25° C.—The procedure was the same as in 3. The spewing was less pronounced, however, and the weighings were sufficiently reliable to be presented in table 1. The comparatively large loss in weight of the unwashed dirty eggs was due to the mechanical loss of material from the surface of the shell.

7. Naturally dirty eggs, holding temperature 20° to 25° C.—This experiment was similar to 4, except for the holding temperature.

The percentage loss in weight of the various lots is given in table 1, and the actual loss in weight in grams for the various time intervals is given in figures 1 to 6. The figures indicate a linear relation between loss in weight and time during the period studied. This is noted especially at 35° C., where the temperature control was better.

No definite effect of washing on the loss in weight is indicated. A definitely retarding effect of oil-dipping on the rate of loss in weight is shown.

The size of the air cells was measured after the eggs had been held for 20 days. The eggs that lost the least weight had the smallest air cells. The eggs held at 35° C. had larger air cells than those held at room temperature. No effect of washing was indicated.

TABLE 1.—*Average percentage loss in weight of eggs cleaned by various methods and cleansing agents*

Cleansing agent and treatment	Clean eggs washed and held at 35° C.		Naturally dirtied eggs washed and held at 35° C. 30 days	Eggs washed and held at room temperature, 20°–25° C.		
	27 days, in cartons	30 days		27 days (clean eggs)	30 days	
					Extremely dirty eggs	Naturally dirtied eggs
Water	6.67	7.60	----------	2.56	2.75	----------
H_2SO_4, N/10	5.57	6.84	----------	2.83	3.28	----------
NaOH, N/10	5.96	7.21	----------	2.90	3.10	----------
Water glass	6.02	6.78	----------	2.68	3.69	----------
Water, oiled	.53	.55	----------	.31	.59	----------
Oiled only	.65	.53	0.58	.41	----------	0.49
Soap	6.21	7.15	6.77	2.80	3.08	4.81
Wyandotte powder	5.42	7.20	----------	2.95	3.44	----------
Sapolio	6.69	7.45	----------	2.98	3.21	----------
Hypochlorite	----------	----------	6.87	----------	3.88	4.42
Sandpaper	----------	----------	6.33	----------	4.28	4.18
Hypochlorite, oiled	----------	----------	.81	----------	----------	.28
Sandpaper, oiled	----------	----------	.54	----------	----------	.70
Soap, oiled	----------	----------	.45	----------	----------	.34
Dirty, unwashed	----------	----------	6.64	----------	5.01	4.77
Clean, unwashed	6.42	7.48	----------	3.22	3.39	----------

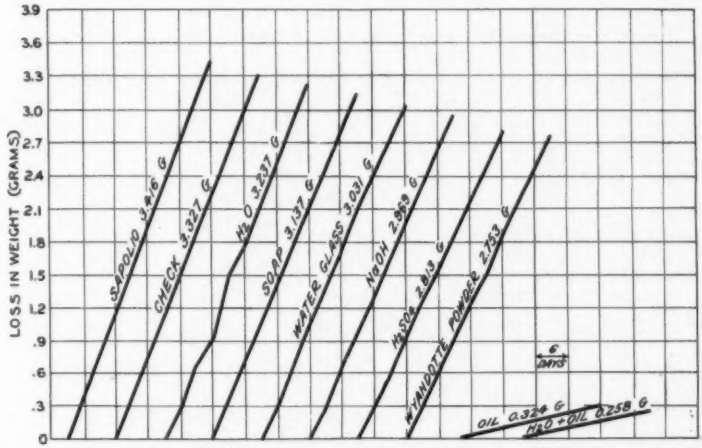


FIGURE 1.—Loss in weight (grams) of clean eggs after being washed with various solutions and held in cartons at 35° C. and 75 percent humidity for 27 days.

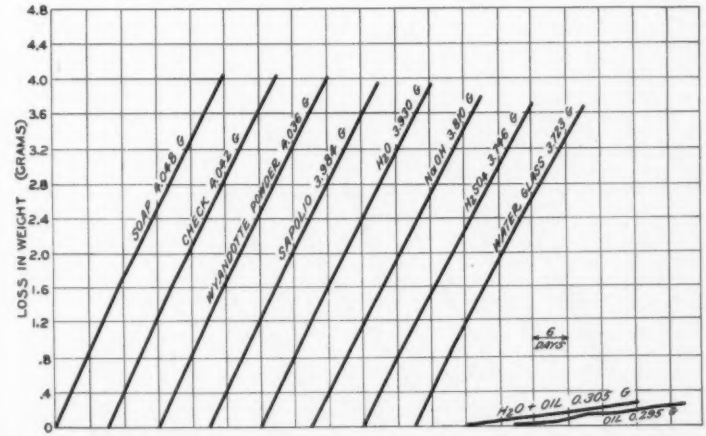


FIGURE 2.—Loss in weight (grams) of clean eggs after being washed with various solutions and held at 35° C. and 75 percent humidity for 30 days.

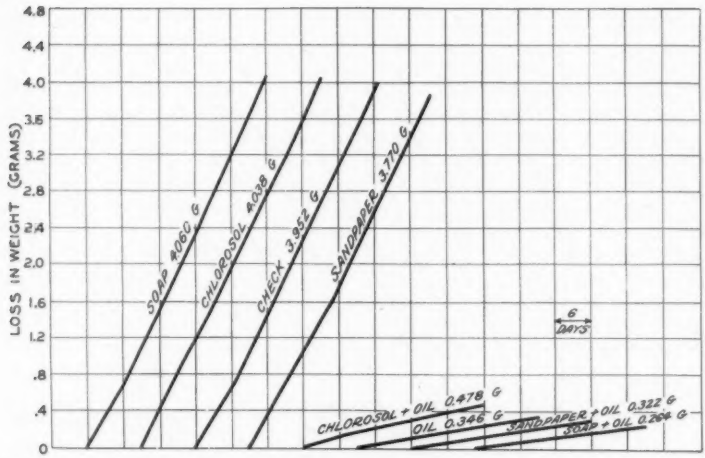


FIGURE 3.—Loss in weight (grams) of naturally dirtied eggs after being washed with various solutions and held at 35° C. and 75 percent humidity for 30 days.

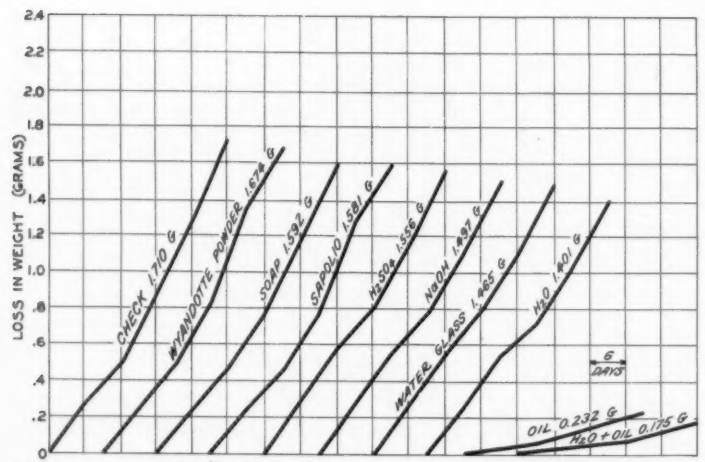


FIGURE 4.—Loss in weight (grams) of clean eggs after being washed with various solutions and held at room temperature (20°–25° C.) and 75 percent humidity for 30 days.

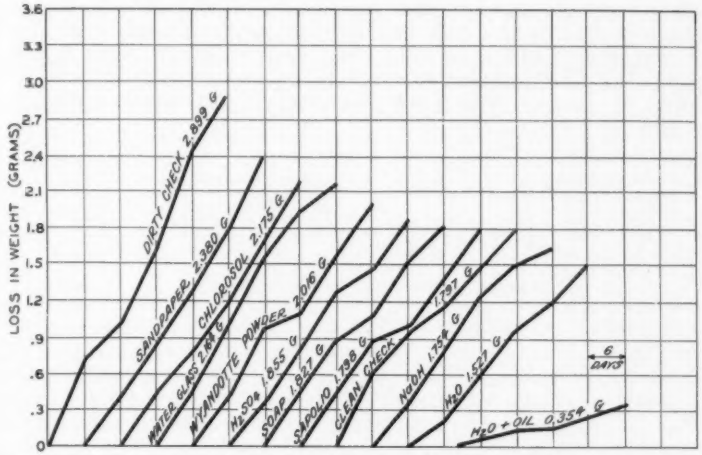


FIGURE 5.—Loss in weight (grams) of extremely dirty eggs after being washed with various solutions and held at room temperature (20°–25° C.) and 75 percent humidity for 30 days.

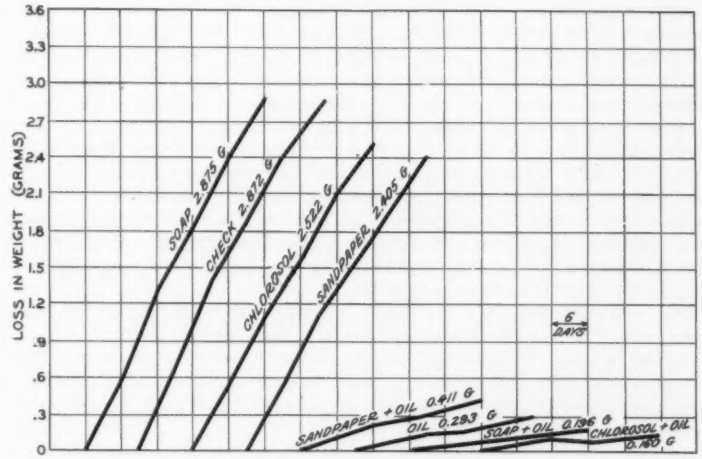


FIGURE 6.—Loss in weight (grams) of naturally dirtied eggs after being washed with various solutions and held at room temperature (20°–25° C.) and 75 percent humidity for 30 days.

Cold Storage

A case of eggs was placed in an egg-storage room of a commercial cold-storage warehouse. The case contained naturally clean eggs, naturally clean eggs which had been washed, naturally dirty eggs, and naturally dirty eggs which had been washed. Each egg was weighed at the beginning of the experiment and after 4.5 and 13.5 months in storage. The mean loss in weight and the probable error for each lot follow:

	Percent
Storage 4.5 months:	
87 clean eggs	2.317± 0.043
88 clean eggs washed	2.315± .036
83 dirty eggs	2.416± .037
89 dirty eggs washed	2.365± .033
Storage 13.5 months:	
84 clean eggs	5.836± .099
86 clean eggs washed	5.866± .089
82 dirty eggs	6.034± .099
88 dirty eggs washed	5.911± .086

The distribution of the eggs was varied throughout the case. This was necessary because often there is considerable variation in the loss in weight of eggs in different layers and in different sides of a case.

The conclusion seems justified that washing in itself has no effect on the rate of loss in weight of eggs in cold storage. The dirty eggs lost more weight than the washed dirty eggs. This difference is insignificant, however, and might have been due to the mechanical loss of the dirt from the eggs.

Removal of Film from Surface of Shell

Eggs were dipped in 10-percent solutions of nitric, hydrochloric, or sulphuric acid for a few seconds, and the protein material thus loosened was rubbed off by washing in tap water. Ten eggs were treated with each acid, held at laboratory temperature with no humidity regulation, and weighed at various intervals. The results obtained are given in table 2. These results indicate that eggs lose weight more slowly after the film is removed. Since the calcium salts formed by the action of nitric and hydrochloric acid on the shell are very soluble, a plugging action due to the formation of insoluble inorganic salts would hardly be expected. Thus it must be concluded that washing eggs does not remove the protecting film from the surface of the shell (*26*), and that if it did the eggs would not lose moisture faster than if it were present.

The results presented in table 2 have been confirmed by other experiments. It seems possible that the material which was removed from the surface of the egg might, when present, act as the outer end of a capillary wick passing between the crystals of the shell to draw moisture from the egg and to increase the evaporating surface.

TABLE 2.—*Effect of washing eggs with 10 percent solutions of nitric, hydrochloric, and sulphuric acids on the percentage loss in weight when held at 20°–25° C. for varying periods*

[This treatment removes a film from the surface of the egg. Each value is the average of the results obtained with 10 eggs]

Time (days)	Percentage loss in weight of eggs				Time (days)	Percentage loss in weight of eggs			
	Not washed	Washed with—				Not washed	Washed with—		
		HNO_3	HCl	H_2SO_4			NHO_3	HCl	H_2SO_4
3	1.26	1.12	1.10	1.13	14	4.74	4.31	4.29	4.39
6	2.12	1.90	1.88	1.94	21	6.73	6.20	6.18	6.26
11	3.72	3.35	3.35	3.44	32	9.65	9.02	8.95	8.92

USE OF THE SAND BLAST

For some years the sand blast has been utilized for cleaning eggs. This method does not involve the wetting of the shell. In some of the experiments described above, eggs were cleaned with sandpaper in an attempt to simulate cleaning with a sand blast. Contrary to expectation, the sandpapered eggs did not differ greatly in rate of loss of water from those that were not sandpapered. This result was obtained in spite of the fact that the sandpapering increased the number of pores, as determined by the water-vacuum method.

Some information as to the effect of the commercial sand blast on the number of pores and the loss in weight of eggs was obtained. Two dozen eggs which had been through the sand blast and 2 dozen similar eggs which had not been sand-blasted were kept in the laboratory at 20° to 25° C., with no humidity regulation, for 4 weeks. The eggs were weighed individually at weekly intervals. The experiment was carried out in the winter, when the humidity of the laboratory was rather low. At the end of 4 weeks the number of pores, as indicated by the appearance of a continuous stream of bubbles when the eggs were immersed in water and a suction pump applied, was determined. The average number of pores on the eggs which were not sand-blasted was 18, while the average number on the sand-blasted eggs was 53. Most of the pores on the sand-blasted eggs were small, however, if the rate of escape of air may be taken as an indication of size. Table 3 shows the average loss in weight of the two lots of eggs.

TABLE 3.—*Percentage loss in weight of sand-blast-treated eggs as compared with untreated eggs when held at 22°–25° C. for from 1 to 4 weeks*

Time (weeks)	Percentage loss in weight of eggs—		Increase in loss in weight due to sanding
	Not sanded	Sanded	
1	2.90	3.54	22.05
2	5.78	6.93	20.12
3	8.14	9.66	18.7
4	11.23	13.18	17.4

The sand-blasted eggs lost weight faster than the eggs that were not sand-blasted, but the average increase in the loss in weight was

only about 20 percent, whereas the increase in the number of pores was over 200 percent. Evidently some other property exerted a strong influence on the loss in weight.

This experiment was repeated 2 years later, with essentially the same results. Eggs collected from a single pen of hens were divided into three lots of 2 dozen each, and held for 45 days after treatment at a temperature of 25° to 30° C. and a relative humidity of 75 percent. In this experiment the sanded eggs were put through the sanding machine twice. The losses in weight were as follows: Control, no treatment, 9.4 percent; dirty and sanded, 13.1 percent; clean and sanded, 13.6 percent.

NUMBER OF PORES AND STRENGTH OF SHELL

As it was believed that the number of pores in the shell might affect the loss in weight, the pores were counted in the eggs used for the data in table 1. These pores were determined by immersing an egg in water, creating a vacuum, and counting the sources of bubbles that came from the egg. The average number of pores for each lot in the various experiments is given in table 4.

TABLE 4.—*Average number of pores in shells of eggs listed in table 1 that were cleaned by various methods and cleansing agents*

[Determined by evacuating eggs under water]

Cleansing agent and treatment	Clean eggs washed and held at 35° C.		Eggs washed and held at 35° C. for 30 days		Eggs washed and held at room temperature, 20°–25° C.		
						30 days	
	27 days, in cartons	30 days	Extremely dirty eggs	Naturally dirtied eggs	27 days (clean eggs)	Extremely dirty eggs	Naturally dirtied eggs
H_2O	15	13			10	10	
H_2SO_4 N/10	8	8			8	9	
NaOHN/10	13	22			21	14	
Water glass	6	13			8	11	
Water, oiled	5	5	5		3	4	
Oiled	4	3		7	6	5	7
Soap	7	9		18	11	5	21
Wyandotte powder	6	8			8	11	
Sapolio	9	17			14	10	
Hypochlorite				22		20	17
Sandpaper			46	28		37	32
Hypochlorite, oiled				8			11
Sandpaper, oiled				10			6
Soap, oiled				9			10
Dirty, unwashed				13		18	10
Clean, unwashed	5	7	9		10	13	

Although the results are not wholly consistent, a few points are indicated. The eggs cleaned with sandpaper had more pores than any of those cleaned by other means. In the lots cleaned with sulphuric acid the number of pores was about the same in each experiment, whereas in the other lots the number of pores varied widely in the different experiments. In these experiments the temperature at which the eggs were held had no effect on the number of pores.

To determine whether the various treatments had any effect upon the strength of the shell the apparatus described by Romanoff (*23*) was used. The smallest weight required to crack the shell of each

egg was determined. The average of the weights necessary to break the shells in each lot of the various experiments is given in table 5.

TABLE 5.—*Average weight necessary to break shell of eggs listed in table 1 that were cleaned by various methods and cleansing agents and held for 30 days*

[Breaking strength in kilograms]

Cleansing agent and treatment	Eggs washed and held at 35° C.			Eggs washed and held at room temperature, 20°–25° C.		
	Clean	Extremely dirty	Naturally dirtied	Clean	Extremely dirty	Naturally dirtied
H_2O	5.1			5.5	3.7	
H_2SO_4	5.4			4.7	4.2	
NaOHN/10	5.1			5.4	4.2	
Water glass	4.1			4.7	4.6	
Water, oiled	4.7	4.7		5.4	4.7	
Oiled	4.2		4.0	4.9		4.1
Soap	5.0		4.4	4.5	4.1	4.0
Wyandotte powder	4.4			5.3	4.3	
Sapolio	4.7			4.5	4.4	
Hypochlorite			4.6		4.5	3.9
Sandpaper		4.4	4.1		4.0	4.2
Hypochlorite, oiled			3.8			4.8
Sandpaper, oiled			3.7			3.7
Soap, oiled			3.7			4.1
Dirty, unwashed			3.7		4.5	3.6
Clean, unwashed	4.2	4.1		4.1	3.6	

The results there shown indicate that washing had no effect upon the strength of the shells.

Individual Variations in Loss of Weight

In each lot studied there was always a variation in the amount of water lost by the individual eggs. Several factors which might account for this variation were studied. Since it was impracticable to divide the eggs in each lot in such a way that the average weights would be exactly the same, a study was made to determine whether the size affected the absolute loss in weight of the egg. The size of the egg, fresh weight, was correlated with the loss in weight after the egg had been held for 30 days at 35° C. The correlation coefficient was $+0.082 \pm 0.067$. Dunn (*9*) says, "There is probably a small or loose association between weight and absolute loss of weight." In the present experiment the association was so small that it is unlikely that egg size had any appreciable effect upon the losses of the various lots.

To determine the effect of the pores upon the loss in weight, correlations were made with two groups of eggs. The eggs were stored for 27 and for 30 days at 35° C. In the first group, all of the eggs used in the first experiment (of table 1) except those that were oil-dipped were considered. The correlation coefficient for the 96 eggs was $+0.450 \pm 0.057$. The second group was made up of 60 unwashed, clean eggs. The correlation coefficient was $+0.461 \pm 0.080$. Both of these are significant. It must be taken into consideration that the size of the pore probably influenced the results, and that there were doubtless many minute pores that could not be determined by the method used in this experiment.

Since there was some question as to whether the number of pores affected the strength of the shell, correlations were made upon 60

clean, unwashed eggs that had been held at 35° C. for 30 days and tested for porosity and shell strength. The correlation coefficient between shell strength and number of pores was $+0.258 \pm 0.033$. Although the correlation is small, it is significant and positive.

An examination of the shell by candling after they had been held for a few days at 75 percent humidity, revealed the presence of a large number of spots. It was thought that the number of spots might be indicative of the condition of the eggs, since they were not apparent when the eggs were first laid. The spots were counted on a shell area of a square centimeter at about midway between the two extremes of the egg. It was found that temperature and washing bore no relationship to these spots. In some instances there were more spots on the eggs that were held at 35° C. than on those held at room temperature. In other instances the reverse was true, and it was concluded that the treatment of the eggs did not affect the number of spots present. The number of pores was correlated with these spots. The correlation coefficient was $+0.172 \pm 0.098$.

FACTORS AFFECTING BACTERIAL COUNT IN EGGS

Germicidal Action of Egg White

Sharp and Whitaker (*29*) found that egg white corresponding in pH to the white of fresh eggs (7.6 to 8.7) was not germicidal, while egg white corresponding in pH to the white of aged eggs (9.5) was germicidal. All of their tests on the germicidal action of egg white were made at 37° C., and the experiment was concluded at the end of 6 hours. In the experiments reported here the germicidal action of egg white was studied at several temperatures and up to 8 days time.

A series of test tubes was arranged, containing equal volumes of well-mixed egg whites sufficient to fill the tubes to about one half of their capacity. The pH of the whites of one half of the tubes was adjusted to 7.48, and that of the other half to 9.56. One cubic centimeter of a broth culture of *Pseudomonas pyocyanea* was added to each tube. The tubes were closed with rubber stoppers in order to prevent the entrance or escape of carbon dioxide, which would have caused a change in the hydroxyl-ion concentration. Tubes containing egg white of each of the pH values were placed at temperatures of 0°, 7°, 16°, 20°, 35°, and 40° C. At various intervals a tube of each was removed, the bacteria plated, and the pH values determined. The results are shown in table 6.

From table 6 it appears that when the pH value is high, germicidal action takes place at all temperatures, but most rapidly at the higher temperatures. When, owing to the production of acid by the bacteria, the pH is lowered, the germicidal action becomes less and the organisms begin to multiply. This multiplication of bacteria began after about 1 day at the higher temperatures, and by the end of the fourth day the pH had dropped to about 6.2 at 20° and 35° C. At the end of 8 days, the bacterial count of the egg white stored at 0° was still decreasing.

At the low pH (7.48) the organisms, after a slight period of lag, multiplied at all temperatures except 0°, and were able to maintain themselves at this temperature. The pH values dropped slightly, but they did not go low enough in 8 days to affect bacterial growth materially.

TABLE 6.—*Effect of pH 9.56 and 7.48 of egg white on growth of pseudomonas pyocyanea (number per cubic centimeter) at different temperatures and at various intervals*

[000 omitted in bacteria for pH 9.56 and 000,000 for pH 7.48]

Temperature (°C.)	Bacteria at start	Bacteria after—							Bacteria after 1 day	pH after 1 day	Bacteria after 2 days	pH after 2 days	Bacteria after 4 days	pH after 4 days	Bacteria after 8 days	pH after 8 days
		½ hour	1 hour	2 hours	3 hours	4 hours	6 hours	8 hours								
	pH 9.56															
0	2,000	.....	900	1,030	....	930	.....	1,200	290	9.57	40	9.54	7	9.54	0.4	9.65
7	2,000	.....	1,090	1,590	....	910	.....	1,070	610	9.43	213	9.50	112	9.52	660	9.45
16	2,000	.....	1,100	1,840	....	1,115	.....	1,310	790	9.43	1,660	9.54	8,300	9.29	118,000	9.13
20	2,000	.....	1,010	930	....	680	.....	270	94	9.43	40,000	9.47	60,000	6.13	(a)	6.21
35	2,000	940	740	850	300	200	100	83	89	9.54	35,000	9.38	75,000	6.22	(a)	6.16
40	2,000	1,070	810	420	240	88	77	75	69	9.53	590	9.47	8,100	9.16	.....	8.98
	pH 7.48															
0	3.8	.....	4.2	3.5	....	2.0	.....	2.7	2.0	7.74	2.6	7.59	3.7	7.63	3.8	7.64
7	3.8	.....	4.5	3.9	....	2.1	.....	2.0	4.4	7.70	9.0	7.65	3.7	7.69	12.0	7.31
16	3.8	.....	3.9	3.8	....	4.1	.....	4.5	5.7	7.69	17.0	7.64	121.0	8.50	400	6.95
20	3.8	.....	4.8	3.7	....	6.9	.....	8.0	35.0	7.70	134.0	7.44	510.0	6.41	670	6.14
35	3.8	3.3	5.7	5.3	6.6	8.1	8.5	7.7	130.0	7.45	760.0	6.49	260.0	6.00	900	b 7.86
40	3.8	4.5	6.2	5.8	4.7	7.2	14.5	10.0	51.0	7.66	310.0	6.59	360.0	5.91	97.0	5.67

[a] Countless.
[b] Stopper had blown out.

METHOD OF CLEANING

A study was made of the number of eggs that contained bacteria in each lot of the various experiments recorded in table 1. For this study, samples of the whites and of the yolks were plated on 0.5-percent glucose agar and the plates incubated 48 hours at 35° C. Since it was necessary to save the whites and yolks for other determinations, the bacteriological sampling technic was not ideal. And egg was not listed as infected unless the plate count was over 100 colonies per cubic centimeter. The percentage of eggs that contained bacteria in the whites and in the yolks are shown in table 7.

It is apparent that only a small percentage of the eggs that were clean before washing contained bacteria, regardless of the method used in washing them. Nearly all of the extremely dirty eggs contained bacteria in both whites and yolks, except the lot that was cleaned with sandpaper, in which some of the eggs were free from micro-organisms. In nearly every lot of naturally dirtied eggs some eggs contained bacteria, but the percentage of eggs having infected whites was no greater in the washed than in the unwashed eggs. The yolks were infected in a larger percentage of cases than the whites. One hundred percent of the yolks of the untreated naturally dirtied eggs contained bacteria; none of the other lots showed as many.

TABLE 7.—*Percentage of eggs listed in table 1 that contained bacteria in whites or yolks after being cleaned by various methods and cleansing agents*

WHITES

Cleansing agent and treatment	Eggs washed and held at 35° C.				Eggs washed and held at room temperature, 20°–25° C., for 30 days		
	27 days, clean, in cartons	30 days			Clean	Extremely dirty	Naturally dirtied
		Clean	Extremely dirty	Naturally dirtied			
Water	0	0	100		0	60	
H_2SO_4 N/10	0	0	100		0	100	
NaOH N/10	0	0	100		0	100	
Water glass	0	0	100		0	100	
Water, oiled	0	0	100		0	100	
Oiled	0	0		33	0		0
Soap	9	8	100	0	8	100	25
Wyandotte powder	8	0	100		0	100	
Sapolio	0	0	100		0	100	
Hypochlorite			100	50		100	50
Sandpaper			50	50		33	20
Hypochlorite, oiled				33			67
Sandpaper, oiled				17			25
Soap, oiled				50			40
Dirty, unwashed			100	40		50	67
Clean, unwashed	0				0		

YOLKS

Water	18	0	100		0	40	
H_2SO_4 N/10	8	0	100		0	100	
NaOH N/10	9	0	100		10	100	
Water glass	8	0	100		0	100	
Water, oiled	0	0	100		0	100	
Oiled	0	0		60	17		42
Soap	9	0	100	60	17	100	33
Wyandotte powder	8	0	100		20	100	
Sapolio	0	0	100		0	100	
Hypochlorite			100	75		100	67
Sandpaper			50	80		33	40
Hypochlorite, oiled				67			67
Sandpaper, oiled				58			25
Soap, oiled				50			50
Dirty, unwashed			100	100		83	100
Clean, unwashed	0				0		

Some of the solutions used in washing the eggs were selected because of their germicidal activity, so that the bacteria which were in the soiled material on the surface of the shell might be killed during the washing process. The hypochlorite solution was one of the most active germicides used. One dozen eggs that had been dipped in a manurial compost were washed in a 1:100 solution of the hypochlorite. As soon as the washing was completed, a sample of the dirty solution was plated on agar. At stated intervals thereafter, samples were plated for a bacterial count. The results of this test are shown in the following tabulation:

Time	*Bacteria per cubic centimeter*
At start	30,000
Half minute	25,600
1 minute	20,000
2 minutes	16,800
5 minutes	12,200
15 minutes	10,900
30 minutes	3,000
1 hour	2,500
2 hours	3,100
4 hours	1,800
8 hours	0

When the washing was completed, there were only 30,000 bacteria per cubic centimeter present, and before the end of 8 hours all of these were dead. It is probable that the vegetative cells were all destroyed by the time the washing was finished, and that the 30,000 bacteria represented only the more resistant spores.

This experiment shows the difficulty of destroying all of the bacteria in the water while the eggs are being washed.

In order to lessen the time required for the experiments the eggs were stored at 35° and at 22° to 25° C. To determine whether such experiments would reflect the results that would be obtained at a lower temperature, an experiment was made in which the eggs were stored for 5½ months at 2°. In this experiment naturally dirtied eggs were used. Four different treatments as shown in table 8 were studied.

TABLE 8.—*The growth of bacteria in 3 dozen naturally dirtied eggs receiving each treatment shown and then stored 5½ months at 2° C. and for 3 additional weeks at 25° and then examined for bacteria*

[One half the eggs were examined at the end of the 5½ months and the remainder after the 3 additional weeks]

Treatment	Average loss in weight	Eggs showing bacteria at end of 5½ months cold storage at 2° C.	Eggs showing bacteria at end of 5½ months cold storage at 2° C. followed by 3 weeks at 25°
Naturally dirtied eggs:	*Percent*	*Percent*	*Percent*
Control, no treatment		11	[a]41
Oil-dipped	0.7	0	11
Washed with Sapolio	3.7	[b]20	[b]27
Washed with Sapolio and oil-dipped	.9	[a]0	[a]12

[a] 17 eggs in the experiment.
[b] 15 eggs in the experiment.

Fewer dirty eggs developed bacterial infection at the low than at the two higher temperatures. However, attention should be called to the fact that the eggs used in this experiment were not an aliquot of those used to obtain the results for naturally dirtied eggs reported in table 7. Infection was less pronounced in the oil-dipped eggs than in those not oil-dipped. This finding cannot be accepted as general, however, for under some conditions infection seems to develop more readily in the oil-dipped eggs.

EFFECT OF WASHING ON DIFFERENT EGG CHARACTERS

Dry-Matter Content

Determinations were made of the percentage of dry matter in the whites and in the yolks of the individual eggs listed in table 1. The white loses water to the air by evaporation and to the yolk by osmosis.

The oil-dipped eggs contained less dry matter in the white and more dry matter in the yolk than did the eggs which were not oil-dipped. This is in agreement with the previously demonstrated effect of oil-dipping on the pH values and the passage of water from the white to the yolk. No effect of washing on this change in dry-matter content was indicated.

Flattening of Yolk

As an egg ages, the yolk takes up progressively more and more water from the white, and when the egg is broken into a dish, the tendency of the yolk to flatten increases until in extreme cases the egg cannot be broken without the yolk membrane breaking. The flattened condition can be expressed in numerical units by dividing the height by the width. This factor obtained from eggs on the day they are laid is about 0.41. The application of this method has been described by Sharp and Powell (*28*). Such measurements were made on the yolks of the eggs of table 1, held at room temperature.

No difference in yolk quality between the washed and unwashed eggs as indicated by this ratio was noted. The value for the oil-dipped eggs was about 0.400, which is only slightly lower than that for fresh eggs. The value for the eggs receiving the other treatments was very low and many yolks broke. The difference between the oil-dipped eggs and the eggs receiving the other treatments was due to the retention of the carbon dioxide.

The extremely dirty eggs were so weak or so badly decomposed that it was possible to obtain measurements on a few yolks only. The values for naturally dirtied eggs were somewhat lower than those for clean eggs. In this case, however, there was nothing in favor of the unwashed eggs over the cleaned eggs. The values were not affected by the cleaning before oiling treatment as compared with oiling without cleaning.

Hydrogen-Ion Concentration

A number of workers (*2*, *3*, *11*, *13*, *27*) have shown that the hydrogen-ion concentration of the egg decreases as it gets older, owing to a loss of carbon dioxide, the pH value of the white increasing from about 7.6 to 9.5 and that of the yolk from 6.0 to 6.8.

The pH values of the whites and yolks of the eggs referred to in table 1 was determined. No effect of the washing treatments could be demonstrated. Many of the eggs which were infected with bacteria showed abnormal pH values. The pH values of the oil-dipped eggs were much lower than those of the eggs that were not oil dipped.

Appearance

In appearance the eggs washed with Sapolio or Wyandotte powder were very much like clean, unwashed eggs; those cleaned with soap were decidedly shiny in appearance and slightly velvety to the touch. The eggs cleaned with acid or alkali had a mottled appearance; those dipped in oil were shiny.

Internally, the oil-dipped eggs were far superior to the others, and those that were washed before they were dipped were the equal of those that had not been washed before they were dipped. The mere washing of the eggs did not seem to cause them to break down at a more rapid rate.

DISCUSSION

This investigation indicates that with the solutions used, the washing of eggs caused no increase in rate of loss of weight as compared with that of unwashed eggs. It does show that the use of abrasive materials, such as sandpaper and sand, may cause a slight increase in the rate of evaporation.

The experiments here recorded show an approximately linear relationship between time and the evaporation of water from eggs at constant humidity. The loss in weight, so far as the writers have followed it did not yield a parabolic curve such as was obtained by Greenlee (*10*). It is obvious, however, that if enough water is permitted to evaporate from the eggs, the rate of loss in weight must eventually decrease. Under conditions of commercial storage the parabolic form of the loss-in-weight curve might be obtained, for during the early period of storage, the fillers and cases absorb moisture from the eggs, whereas toward the end of the storage period the fillers and cases are more nearly in equilibrium with the humidity of the atmosphere, and consequently the loss in weight of the eggs is not so rapid.

The more recent investigations of Almquist and Holtz (*4*) have created some uncertainty as to the best method for determining porosity. The method recommended by them consists in immersing eggs in an alcoholic solution of methylene blue for 2 minutes, then removing the eggs and opening them. The porosity is indicated by the number of blue spots on the inside of the shell, and is expressed in terms of a set of standard shells showing varying degrees of staining. Almquist and Holtz found that loss in weight increased with porosity, as determined by their method, but they did not give the correlation coefficients. They state that the eggs were held at constant humidity, but they do not state what humidity was used. From the great loss in weight reported, they must have used a very low hunidity, which may account for their results.

Almquist and Holtz have criticized the method used in the present experiments for determining pores, intimating that the changes in pressure would open the pores. This criticism is hardly justified by the facts. The number of pores was repeatedly determined on a series of eggs at weekly intervals for 6 weeks by the water-immersion vaccum method. While it is true that the number of pores increased or decreased slightly with some eggs, this change in number was usually within the possible error of counting, and, on the whole, the change in number was not nearly so great as the increase in porosity which Almquist and Holtz report as having occurred without any vacuum or pressure treatment at all.

This great increase in porosity with time, as determined by the Almquist and Holtz method, is rather puzzling, for if this increase in porosity is significant the eggs should lose weight at an increasing rate with time of holding. This does no appear to be the case. Minor factors, such as increase in osmotic pressure and air cell, could not

counterbalance the effect, since the increase in porosity is often many times that of the freshly laid egg.

While the water-immersion vaccum method for determining pores leaves much to be desired, yet within fairly narrow limits, the method yields the same results whether the porosity is determined soon after the eggs are laid or after they have been stored. This agrees more nearly with the fairly constant loss in weight of the eggs under constant conditions. The pores which are shown on normal eggs are true channels through the shell as can be readily seen by examining them under a relatively low-power microscope. The significance of the water-immersion vacuum method may also be questioned because sanded eggs show a great increase in porosity and the increased rate of loss in weight is not in proportion.

Attention should be called to the importance of the alcohol in the Almquist and Holtz method. If water is used as a solvent, much the same porosity is indicated as is obtained with the water-immersion vacuum method; if alcohol is used as a solvent, the indicated porosity is much greater. A preliminary treatment of the eggs with alcohol does not increase the porosity as determined by the water-immersion method, however.

Stewart,[3] working in this laboratory, obtained about the same correlation between loss in weight and shell thickness as the writers obtained between porosity and loss in weight.

A relation between the number of pores and the strength of the shell, as indicated by the correlation coefficient $+0.258 \pm 0.033$, was found. This indicates that up to a certain point the stronger the shell, the greater the number of pores. If, however, the shell is too porous the strength is lessened.

More extensive experiments have been in progress for some time in an effort to gain information on the factors involved in the loss in weight. While the evidence still points to the importance of the pores as one factor under some conditions the relation between the number of pores and the loss in weight is not as definite as was found in these experiments.

It seems reasonable to assume that bacteria and molds enter the egg by way of the large pores. Pennington[4] has shown that mold growth on the inside of an egg begins at the large pores. Since the large pores are relatively few in number, an egg might be soiled without the material actually covering a pore. Such eggs might escape infection. If the dirt happened to cover a portion of the shell containing a pore, the chance of infection would probably be greater. It has been assumed that the washing operation would spread the infected material over the surface of the eggs and increase the chances of some of it entering the pores. Jenkins, Hepburn, Swan, and Sherwood (*14*) have shown that washing dirty eggs increased the number that showed bacterial infection during storage. The data herein presented are too meager to warrant a definite conclusion on this point.

Rettger (*21*), Hadley and Caldwell (*12*), and others have demonstrated that very few clean newly laid eggs contain bacteria. The writers' observations confirmed this finding; very few such eggs showed infection even after being held at 35° C. for 30 days. On the

[3] STEWART, G. F. SHELL CHARACTERISTICS AND WEIGHT LOSSES OF HEN'S EGGS. 1933. (Unpublished.)
[4] PENNINGTON, M. E. Private communication.

other hand, eggs that had been heavily coated with manure and allowed to dry overnight contained bacteria after storage, regardless of the means of washing employed. A large number of the eggs that had been taken from the nest as "dirties" were found to contain bacteria after 30 days of storage. The washing of dirty eggs did not seem to increase greatly the number which were found to contain bacteria at the end of the storage period.

The humidity of the air in which the eggs are kept is a very important factor in determining whether dirty or washed dirty eggs will show the greatest spoilage.

These experiments indicate that washing naturally clean eggs with clean water and germicidal solutions has little or no effect upon the keeping quality of the eggs. This conclusion appears to be in opposition to the current belief that washing in itself causes eggs to deteriorate. Washed dirty eggs, however, do not keep so well as naturally clean eggs. The main reason for the deterioration of washed eggs commercially rests in the fact that the eggs were dirty before they were washed, and consequently the chance of bacterial infection was greatly increased. The number of washed or cleaned eggs which will show bacterial spoilage will depend upon the condition of the eggs before they were washed or cleaned and upon their treatment.

Some incidental observations on the effect of cleaning dirty eggs by abrasion indicate that this method does not insure against bacterial infection.

SUMMARY AND CONCLUSIONS

Eggs washed with a number of different solutions did not lose weight during storage at high temperatures more rapidly than unwashed eggs.

Oil-dipped eggs showed a smaller loss in weight during storage than eggs that were not oil-dipped.

The loss in weight was at a uniform rate for the length of the experimental period, 30 days.

A significant, positive correlation ($+0.450 \pm 0.057$) between the number of pores in the eggshell and the loss in weight of the egg was found.

A small positive correlation ($+0.258 \pm 0.033$) between the number of pores and the breaking strength of the shell was found.

Attention is called to the possibility that infection from bacteria may take place through the relatively few large pores of the shell.

The germicidal action of egg white having a high pH value is evidenced at temperatures from 0° to 40° C.

From the results here reported it is concluded that there is no foundation for the common belief that washing in itself causes eggs to deteriorate, if they are properly handled after washing. The deterioration of washed eggs is caused by bacterial infection of the egg from the dirt that was on the shell. The only effective remedy is to prevent the eggs from becoming dirty.

LITERATURE CITED

(1) ANONYMOUS.
1928. DIRTY EGGS. Reliable Poultry Jour. 34: 804.

(2) AGGAZZOTTI, A.
1913. INFLUENCE DE L'AIR RARÉFIE SUR L'ONTOGENÈSE. NOTE II. I^re^ PARTIE. LA RÉACTION DES LIQUIDES DE L'ŒUF DURANT LE DEVELOPPEMENT DANS LA PLAINE. Arch. Ital. Biol. 59: [305]–321.

(3) AGGAZZOTTI, A.
1914. INFLUENZA DELL'ARIA RAREFATTA SULL'ONTEGENESI. NOTA III. LE MODIFICAZIONI CHE AVVENGONO NEIGAS DELLA CAMERA D'ARIA DELL'OVO DURANTE LO SVILUPPO. Arch. Entwickl. Mech. Organ. 40: [65]–97, illus.
(4) ALMQUIST, H. J., and HOLST, W. F.
1931. VARIABILITY OF SHELL POROSITY IN THE HEN'S EGG. Hilgardia 6: [61]–72, illus.
(5) BENJAMIN, E. W.
1923. MARKETING POULTRY PRODUCTS. 328 pp., illus. New York.
(6) BUSHNELL, L. D., and MAURER, O.
1914. SOME FACTORS INFLUENCING THE BACTERIAL CONTENT AND KEEPING QUALITY OF EGGS. Kans. Agr. Expt. Sta. Bull. 201, pp. [749]–777.
(7) CURTIS, M. R.
1914. FACTORS INFLUENCING THE SIZE, SHAPE AND PHYSICAL CONSTITUTION OF THE EGG OF THE DOMESTIC FOWL. Maine Agr. Expt. Sta. Bull. 228, pp. 105–136.
(8) DUNN, L. C.
1922. THE VARIATION OF EGGS IN THE RATE AT WHICH THEY LOSE WEIGHT. Poultry Sci. 2: [45]–58.
(9) ———
1923. THE VARIATION OF EGGS IN THE RATE AT WHICH THEY LOSE WEIGHT. PART II. THE RELATIONSHIP BETWEEN THE SIZE AND THE LOSS OF WEIGHT OF EGGS. Poultry Sci. 2: [166]–171.
(10) GREENLEE, A. D.
1911. DETERIORATION OF EGGS AS SHOWN BY CHANGES IN THE MOISTURE CONTENT. U.S.Dept.Agr., Bur. Chem. Circ. 83, 7 pp., illus.
(11) GUEYLARD, F., and PORTIER, P.
1925. RÉACTION IONIQUE DES DIFFÉRENTS CONSTITUANTS DE L'ŒUF DE LA POULE. LES MODIFICATIONS AN COURS DE L'INCUBATION. Compt. Rend. Acad. Sci. [Paris] 180: 1962–1963.
(12) HADLEY, P. B., and CALDWELL, D. W.
1916. THE BACTERIAL INFECTION OF FRESH EGGS. R.I.Agr.Expt.Sta. Bull. 164, 70 pp., illus.
(13) HEALY, D. J., and PETER, A. M.
1925. THE HYDROGEN ION CONCENTRATION AND BASICITY OF EGG YOLK AND EGG WHITE. Amer. Jour. Physiol. 74: 363–368.
(14) JENKINS, M. K., HEPBURN, J. S., SWAN, C., and SHERWOOD, C. M. S.
1920. EFFECT OF COLD STORAGE ON SHELL EGGS. Ice and Refrig. 58: 140–147, illus.
(15) JENKINS, M. K., and PENNINGTON, M. E.
1919. COMMERCIAL PRESERVATION OF EGGS BY COLD STORAGE. U.S. Dept. Agr. Bull. 775, 36 pp., illus.
(16) JULL, M. A.
1924. EGG WEIGHT IN RELATION TO PRODUCTION. PART I. THE RELATIONSHIP OF THE WEIGHTS OF THE PARTS OF THE EGG TO THE TOTAL EGG WEIGHT. Poultry Sci. 3: 77–78.
(17) LASCHTSCHENKO, P.
1909. ÜBER DIE KEIMTÖTENDE UND ENTWICKLUNGSHEMMENDE WIRKUNG VON HÜHNEREIWEISS. Ztschr. Hyg. 64: [419]–427.
(18) MAURER, O.
1911. BACTERIOLOGICAL STUDIES ON EGGS. Kans. Agr. Expt. Sta. Bull. 180, pp. 333–396, illus.
(19) PENNINGTON, M. E.
1910. A CHEMICAL AND BACTERIOLOGICAL STUDY OF FRESH EGGS. Jour. Biol. Chem. 7: 109–132.
(20) ——— and PIERCE, H. C.
1911. THE EFFECT OF THE PRESENT METHOD OF HANDLING EGGS ON THE INDUSTRY AND THE PRODUCT. U.S.Dept.Agr. Yearbook 1910: 461–476.
(21) RETTGER, L. F.
1913. THE BACTERIOLOGY OF THE HEN'S EGG, WITH SPECIAL REFERENCE TO ITS FREEDOM FROM MICROBIC INVASION. Conn. (Storrs) Agr. Expt. Sta. Bull. 75, pp. [291]–213.

(22) RETTGER, L. F., and SPERRY, J. A.
1912. THE ANTISEPTIC AND BACTERICIDAL PROPERTIES OF EGG-WHITE. Jour. Med. Research (n.s. 21) 26: 55–64.
(23) ROMANOFF, A. L.
1929. STUDY OF THE PHYSICAL PROPERTIES OF HEN'S EGGSHELL IN RELATION TO THE FUNCTION OF SHELL-SECRETORY GLANDS. Biol. Bul. 56: 351–356, illus.
(24) SCHOLL, H.
1893. BACTERIOLOGISCHE UND CHEMISCHE STUDIEN ÜBER DAS HÜHNEREIWEISS. Arch. Hyg. 17: [535]–551.
(25) SHARP, P. F.
1929. THE pH OF THE WHITE AS AN IMPORTANT FACTOR INFLUENCING THE KEEPING QUALITY OF HENS' EGGS. Science (n.s.) 69: 278–280.
(26) ———
1932. DETECTION OF WASHED, ABRASED AND OILED EGGS. Indus. and Engin. Chem. 24: 941–946.
(27) ——— and POWELL, C. K.
1927. PHYSICO-CHEMICAL FACTORS INFLUENCING THE KEEPING QUALITY OF HEN'S EGGS IN STORAGE. World's Poultry Cong., Ottawa, Canada, Rpt. Proc. 27: 399–402.
(28) ——— and POWELL, C. K.
1930. DECREASE IN INTERIOR QUALITY OF HENS' EGGS DURING STORAGE AS INDICATED BY THE YOLK. Indus. and Engin. Chem. 22: 908–910, illus.
(29) ——— and WHITAKER, R.
1927. THE RELATION OF THE HYDROGEN ION CONCENTRATION OF EGG WHITE TO ITS GERMICIDAL ACTION. Jour. Bact. 14: 17–46, illus.
(30) STARK, C. N., and SHARP, P. F.
1927. THE RELATION BETWEEN THE HYDROGEN ION CONCENTRATION OF EGG WHITE AND THE GROWTH OF ANAEROBES. Jour. Bact. 13: 59–60.
(31) STILES, G. W., JR., and BATES, C.
1912. A BACTERIOLOGICAL STUDY OF SHELL, FROZEN, AND DESICCATED EGGS; MADE UNDER LABORATORY CONDITIONS AT WASHINGTON, D.C. U.S.Dept.Agr., Bur. Chem. Bull. 158, 36 pp., illus.
(32) VAN WAGENEN, A.
1930. PRODUCING CLEAN EGGS. Cornell Univ. Dept. Poultry Husbandry, Egg Market Rev. 5 (38): 2–3.
(33) WILEY, H. W., with the collaboration of PENNINGTON, M. E., STILES, G. W., JR., HOWARD, B. J., and COOK, F. C.
1908. A PRELIMINARY STUDY OF THE EFFECTS OF COLD STORAGE ON EGGS, QUAIL, AND CHICKENS. U.S.Dept.Agr., Bur. Chem. Bull. 115, 117 pp., illus.
(34) WURTZ, R.
1890. DE L'ACTION BACTÉRICIDE DU BLANC D'ŒUF. Semaine Med. [Paris] 10: 21.

A MORPHOLOGICAL STUDY OF BLIND AND FLOWERING ROSE SHOOTS, WITH SPECIAL REFERENCE TO FLOWER-BUD DIFFERENTIATION[1]

By D. S. HUBBELL[2]

Assistant horticulturist, Arkansas Agricultural Experiment Station

INTRODUCTION

The formation of nonflowering shoots on forced rose plants represents a great economic loss to rose growers, for in certain varieties this flowerless growth may bring about a 50-percent reduction of each year's potential rose crop.[3]

It is well known that under greenhouse conditions the flowering rose shoot reaches maturity in approximately 45 days, whereas the blind shoot ceases growth after about 30 days and produces a new shoot from the last axillary bud. In the majority of cases this new shoot continues with blind growth for 30 days, at the end of which time it produces a new shoot in the same manner as its parent. The process of flower differentiation in the rose has not yet been established, nor has the author been able to find any report of an anatomical or morphological study of blind and normal rose wood. This paper reports the results of studies made on blind and flowering rose shoots of the variety Briarcliff.

REVIEW OF LITERATURE

The first important work concerning the causes of blindness in roses was done by Corbett.[4] He attributed the production of blind wood to inheritance. The relation of blind-wood production to length of day is being studied by Grove.[5] In a recent report by the present writer,[3] the following results were presented:

A correlation between the physiological behavior and chemical differences of blind and flowering rose shoots indicates that blindness in the rose is a physiological rather than a genetic or pathological condition.

A combination of pruning and budding experiments indicates that blindness is a result of the stock and is not due to impotency of the buds. This point is emphasized by the differences in the chemical composition of blind and flowering wood.

Growth and differentiation were definitely affected by the monthly hours of illumination and the available nitrate supply. A decrease in illumination decreased both flower and blind-shoot production, while the normal increase in illumination in the spring months increased flower production more rapidly than blind-shoot production.

With an increase in soil nitrates blind-shoot formation decreased and flower production increased; with a decrease in soil nitrates blind-shoot formation increased and flower production decreased.

The chemical analyses indicate that blindness is associated with high percentages of noncolloidal nitrogen and insoluble carbohydrates, whereas the flowering shoots contain high percentages of reducing sugars.

[1] Received for publication Aug. 9, 1933; issued February 1934.

[2] The author expresses his appreciation to Prof. J. R. Cooper and Dr. L. M. Turner for suggestions and criticisms throughout the progress of this work.

[3] HUBBELL, D. S. Unpublished thesis, Iowa State College. 1932.

[4] CORBETT, L. C. IMPROVEMENT OF ROSES BY BUD SELECTION, OR BLIND VS. FLOWERING WOOD FOR ROSE CUTTINGS. Mem. Hort. Soc. N.Y. 1: [93]–101, illus. 1902.

[5] GROVE, L. D. Unpublished material, Iowa State College.

Since this work indicated that flower-bud formation is influenced by various cultural practices, a successful interpretation of these influences would depend upon a definite understanding of the time and extent of flower-bud differentiation. In order to obtain such an understanding it would be necessary to make a complete anatomical analysis of the two types of shoots. The purpose of the study here reported was to establish through morphological methods whether blindness in roses is a genetic or a physiological condition. When this point has been established the foundation for the control of blindness in roses will have been laid.

MATERIALS AND METHODS

Beginning December 1, 1932, 10 buds from flowering rose shoots and 10 buds from blind rose shoots were tagged each day until January 4, 1933. Each day the mature stems of blind and flowering stems were selected. The stems were cut back to the buds occupying the fourth axillary position and these buds were collected and studied after they had attained the desired age. The collection consisted of 350 buds and shoots from apparently blind wood and 350 normal buds and shoots. The buds and shoots collected ranged in age from 1 to 35 days.

Immediately after collection the buds and shoots were killed in formalin acetic alcohol. The material was then fixed, cleared, embedded, and sectioned according to the standard method as outlined by Chamberlain.[6] The slides were stained with safranine and light green.

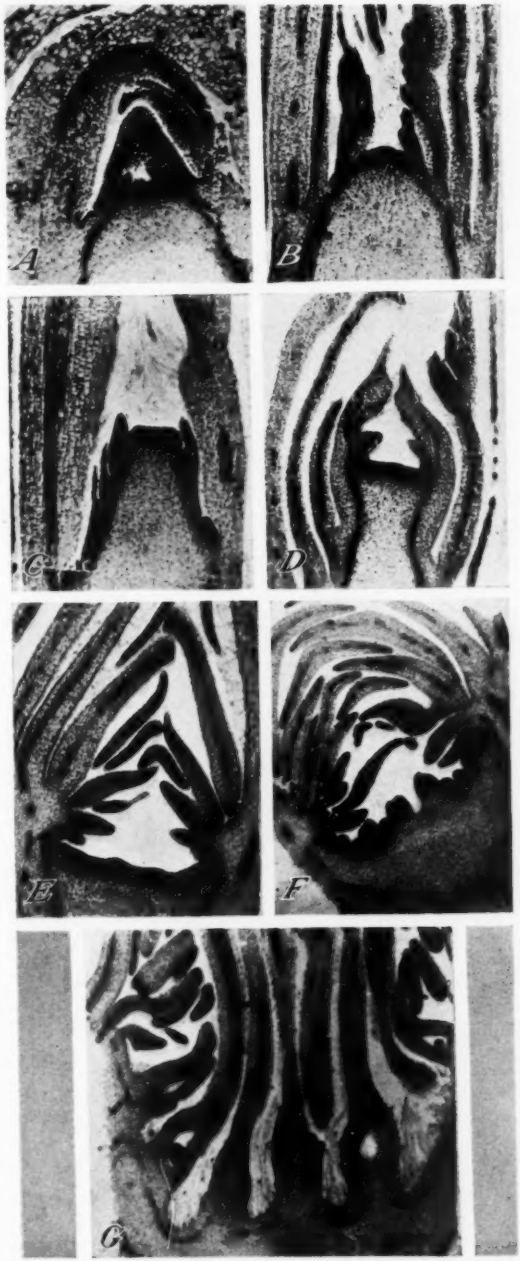
RESULTS

DIFFERENTIATION AND DEVELOPMENT OF FLOWERING SHOOTS

Some differences were noted in the rate of shoot development among buds and shoots of the same age. In general, however, the majority of the buds developed at the same rate for the entire 35 days. Of the shoots and buds gathered, 3.8 percent developed blind wood, 2.8 percent failed to develop it, and 93.4 percent developed normally.

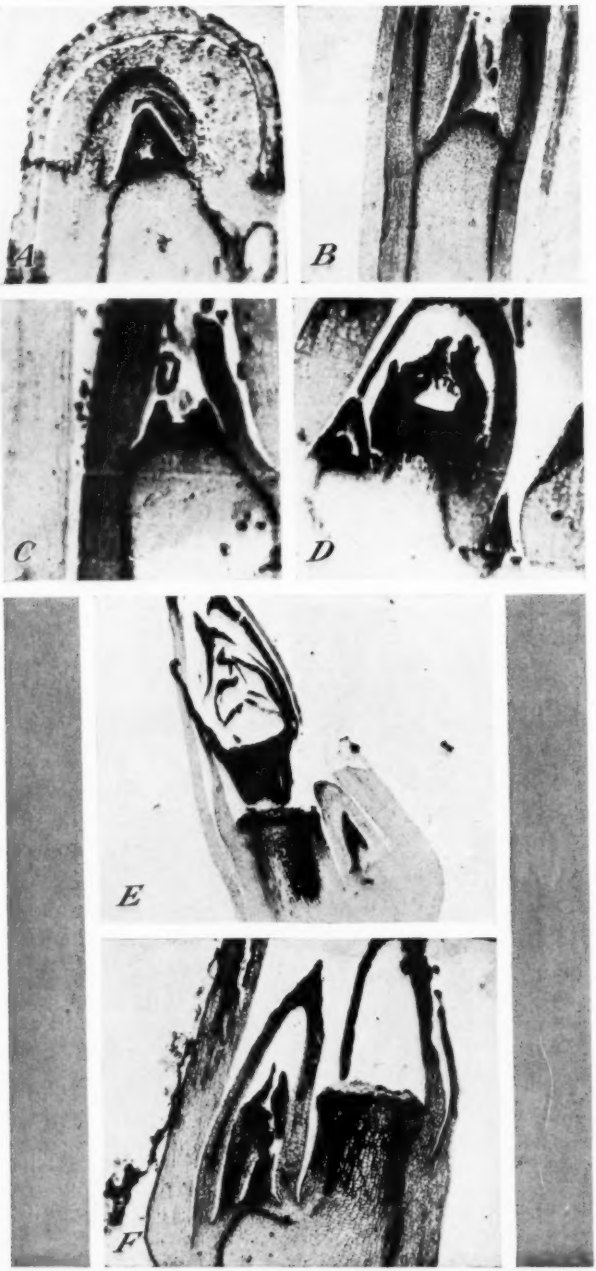
Figure 1 and plate 1 show the development of a normal flowering rose shoot from the purely vegetative bud to the complete formation of the floral parts. The first evidence of differentiation was found 8 days after the axillary bud had been made to assume the terminal position by the removal of the terminal bud (fig. 1, *D*). At this time a broadening and thickening of the floral axis was noted. The ninth day gave evidence of sepal protuberances pushing up from the sides of the bud (fig. 1, *E*). The first stages of petal primordia were observed after the lapse of 12 days (fig. 1, *G*). On December 30, when the bud was 20 days of age pistil primordia appeared as small protuberances on the bottom of the recepticale cup (fig. 1, *J*). At this stage the stamen primordia were also visible, although they were not clearly differentiated into anthers and filaments until the twenty-second day (fig. 1, *K*). By December 25 the stamens and pistils were so well differentiated that their component parts could be readily identified, and the petals had so far developed as to enclose the stamens and pistils (fig. 1, *L*).

[6] CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. 3d rev. ed., 314 pp., illus. Chicago. 1915.



PHOTOMICROGRAPHS OF LONGITUDINAL SECTIONS THROUGH FLOWERING ROSEBUDS AT VARIOUS STAGES OF DEVELOPMENT.

A, Immature bud 1 day old. *B*, 8-day shoot showing the first stage of differentiation. *C*, 10-day shoot showing the formation of sepal primordia. *D*, 12-day shoot with evidence of petal primordia. *E*, 21-day shoot showing pistil protuberances and stamen formation. *F*, 23-day shoot showing advanced stages of pistil primordia. *G*, 25-day shoot showing complete development of all floral organs. × 80.



PHOTOMICROGRAPHS OF LONGITUDINAL SECTIONS THROUGH BLIND ROSE-BUDS AT VARIOUS STAGES OF DEVELOPMENT.

A, Immature bud at the end of the first day. *B*, Signs of differentiation at the end of 10 days. *C*, Sluggish differentiation at 20 days. *D*, Evidence of abortion at 28 days, with petal primordia just beginning to develop. *E*, Evidence of abortion after complete petal formation. *F*, Axillary bud starting active growth after abortion of the terminal bud. × 80.

DEVELOPMENT OF BLIND SHOOTS

The development of the buds taken from blind stems was very irregular, as is shown in plate 2. Of the shoots and buds gathered, 5 percent formed flowers, 10 percent failed to develop beyond the axillary stage, and 85 percent formed blind shoots. The growth rate of the actively growing blind shoots was so lacking in uniformity that

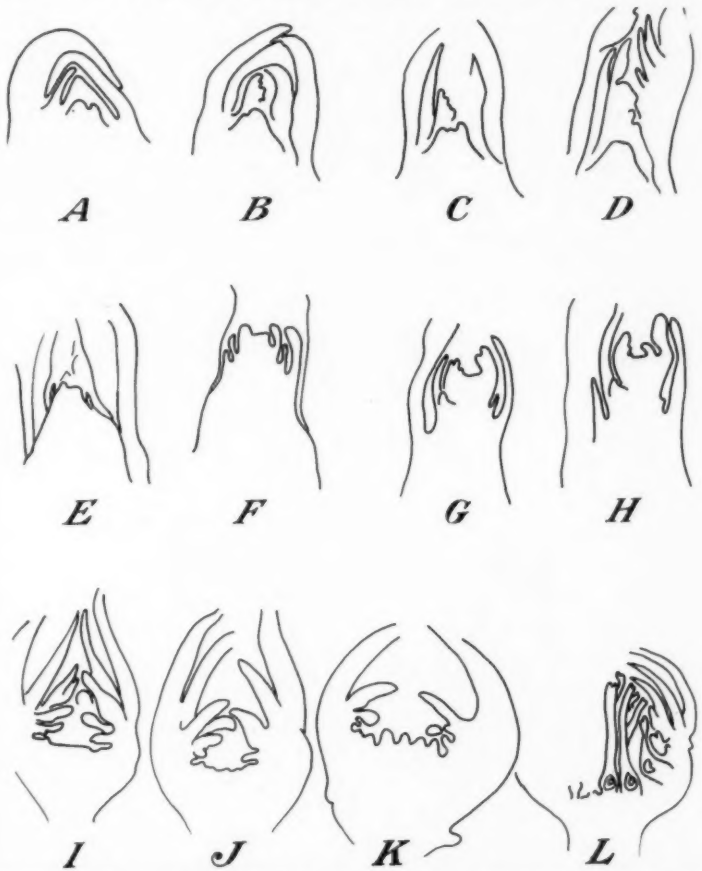


FIGURE 1.—Outline drawings of longitudianl sections through flowering rose-buds, showing the average stages of development from 1 to 25 days: *A*, 1 day; *B*, 3 days; *C*, 5 days; *D*, 8 days; *E*, 9 days; *F*, 10 days; *G*, 12 days; *H*, 14 days; *I*, 18 days; *J*, 20 days; *K*, 22 days; *L*, 25 days. × 50.

no definite dates of differentiation could be determined. Figure 2 shows sections of buds and shoots which displayed the greatest rate of maturity in each day's collection.

Figure 2, *C*, shows the characteristic flattening of the main axis which was the first indication of flower differentiation noted in the flowering shoots. This initial development took place 2 days later in the blind shoots than in the flowering shoots. At the end of the twenty-

fourth day (fig. 2, *E*) the sepal primordia were very prominent, and definite petal primordia appeared from 2 to 6 days later, as is shown in figure 2, *F* and *G*. The formation of petals and sepals, together with a broadening of the receptacle cup, continued until the shoot was 34 days of age. At that time, without any evidence of pistil or stamen

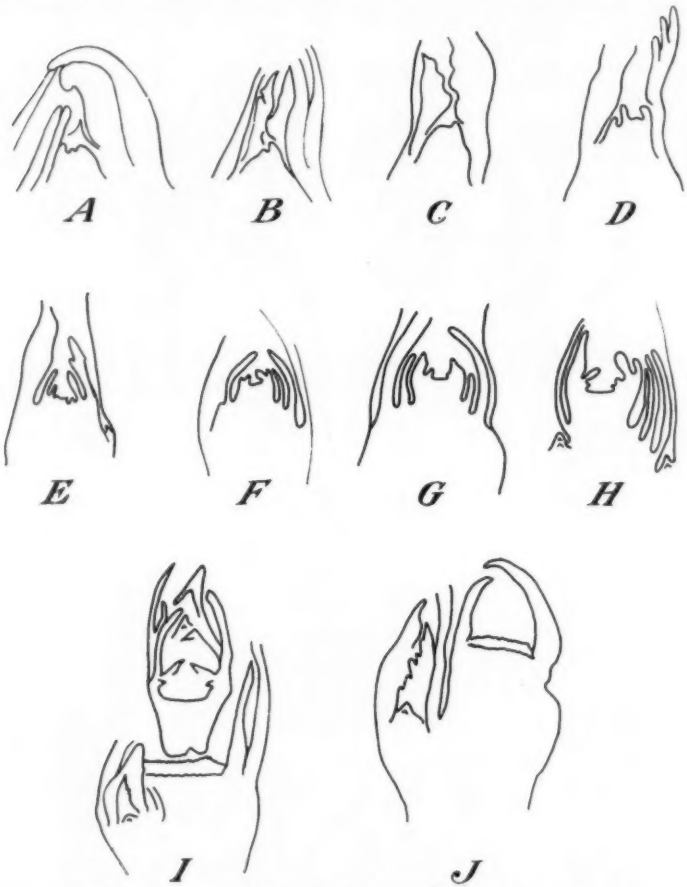


FIGURE 2.—Outline drawings of longitudinal sections through blind rosebuds, showing the average stages of development from 1 to 35 days: *A*, 1 day; *B*, 5 days; *C*, 10 days; *D*, 15 days; *E*, 24 days; *F*, 26 days *G*, 30 days; *H*, 32 days; *I*, 34 days; *J*, 35 days. × 50.

primordia, the bud showed clear signs of abortion (fig. 2, *I*). The condition of the blind shoot after complete abortion of the immature bud is shown in figure 2, *J*. The development of the new shoot, which normally begins growth from the last axillary bud, was in evidence at the end of 32 days, and at the end of 35 days the new shoot had assumed active growth (fig. 2, *I* and *J*).

SUMMARY AND CONCLUSIONS

A study was made of the growth and development of blind and flowering rose shoots with special reference to the date of flower-bud differentiation and the morphological differences between blind and flowering shoots. It was found that the approximate date of flower bud differentiation on flowering shoots was from 8 to 10 days after active growth had started. The complete formation of the flower with all parts completely differentiated was first noted at the end of the twenty-fifth day. Blind shoots were formed when the floral axis failed to develop a complete set of floral organs. Flower-bud differentiation started about 2 days later in the blind shoots than in the flowering shoots. The sepals and petals formed in the blind shoots, but no stamen or pistil primordia appeared. At the end of 30 days the undeveloped flower showed signs of abortion and 5 days later it had completely aborted. Signs of abortion were noted in a few cases at the end of 28 days.

Since the writer [7] has shown that blindness may be controlled by altering nutritional factors, and since abortion is associated with nutrition, it is evident that blindness is purelya physiological condition, in which an abortive bud is formed as a result of an improper balance of nutritional factors.

[7] HUBBELL, D. S. See footnote 3.

U. S. GOVERNMENT PRINTING OFFICE: 1934

Vision